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Experimental and Analytical Studies of Pteridophytes

XXIII. The Induction of Buds in *Ophioglossum vulgatum* L.

BY

C. W. WARDLAW

(*Department of Cryptogamic Botany, University of Manchester*)

With Plates XXVII–XXX and fifteen Figures in the Text

Abstract

A study has been made of the development of buds on isolated root and stem segments of *Ophioglossum vulgatum*. In both cases buds may develop endogenously. The anatomical details of development are fully described. The bearing of the facts on current theories of shoot initiation is discussed.

Introduction

ROOT-BUDS are known to be of general occurrence in *Ophioglossum vulgatum*, *O. pedunculosum*, *O. pendulum*, and other species (Stenzel, 1857; Rostowzew, 1891; Poirault, 1893; Goebel, 1902, 1918; Campbell, 1911; Bower, 1926); they are, in fact, the common means of propagation. Many of these buds originate at or near the horizontally disposed root apex. After giving rise to a bud the root apex may grow on and repeat this process several times, a chain of plantlings being thus formed. Goebel (1902) has shown that detached roots of *O. vulgatum* and *O. pedunculosum* may give rise to buds, and that buds are formed on still-attached roots when the shoot apex is destroyed. Rostowzew (1891) and Poirault (1893) have also described and figured the formation of occasional buds on the root-stock or shoot. These materials would appear to afford an interesting and unusual opportunity of extending our knowledge of morphogenesis and, in particular, of the inception of buds. A reinvestigation of *O. vulgatum* has therefore been undertaken on an experimental basis. This has included observations of (i) the growth and regeneration of plants in pot culture, (ii) the formation of buds in isolated roots and in decapitated shoots, and (iii) the effect of defoliation. The data obtained are described and discussed in this paper.

Materials and Methods

Supplies of *Ophioglossum vulgatum* were obtained from a local source, the root-stocks being typically found at a depth of 6 to 7 in. The plants were carefully lifted, washed, selected for experimental treatment, and planted in pots containing some of the original soil. The plants and isolated roots were

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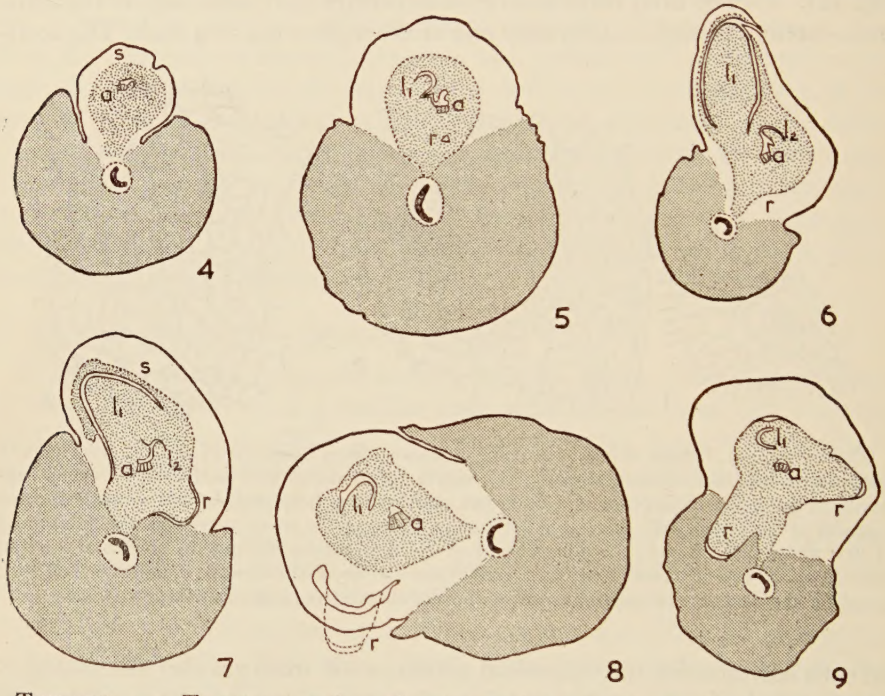
materials of *O. vulgatum*, individual cells of the inner cortex which lie in the direct path between the bud apex and parent root stele being conspicuously affected (Pl. XXVIII, Fig. 15). Cortical cells lateral to the main basipetal strand, and even those at a considerable distance from it, may also be stimulated to divide. In some preparations, localized regions of the pericycle of the parent-root stele had been activated and had divided to form a cambium-like tissue.



TEXT-FIGS. 4–9. Transverse sections of mature regions of roots, showing experimentally induced buds. The bud apex *a*, its first and second leaves, l_1 and l_2, and its root, *r*, are indicated. The endogenous nature of the induced buds is clearly shown, the bud, with its outer sheath, *s*, having been formed from a sector of the cortical parenchyma. An incipient vascular strand traverses the inner cortex between the bud and the root stele. [Figs. 4, 5, 7 (× 20); Fig. 8 (× 25); Figs. 6, 9 (× 12).]

Lastly, it may be noted that these endogenous buds do not arise in any definite relation to the root stele (Text-figs. 4–9).

The endogenous bud enlarges rapidly, and with the formation of a second or third leaf and one or more roots, it bursts through the outer cortical tissues (Pl. XXVII, Fig. 6; Pl. XXIX, Fig. 19; and Text-figs. 4–9). The organization of the shoot apex and the mode of formation of the leaves with their sheathing bases are in close agreement with the published descriptions (Pl. XXIX, Fig. 16). The massive bud roots are a rather striking feature.

Buds which have been formed close to the apex are also of endogenous origin (Pl. XXIX, Fig. 19); as the illustration shows, there is a considerable disruption of the cortex by the emergence of the first leaf. In this specimen,

as also in those illustrated in Pl. XXIX, Figs. 17, 18, the 'sheath' of the first-formed bud leaf has clearly been organized out of the tissues of the middle and outer root cortex.

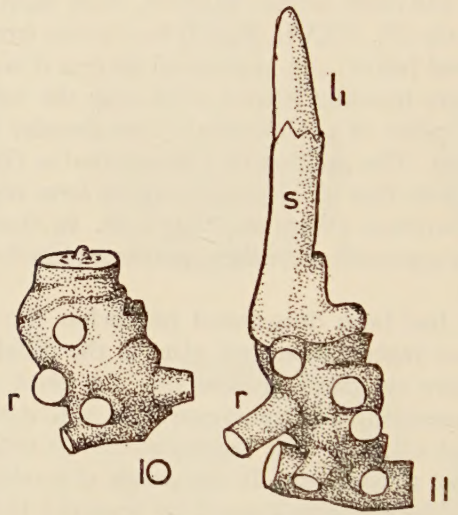
(*c*) *The induction of buds on the shoot*

Lateral shoot buds are occasionally found under natural conditions. The specimen figured by Rostowzew shows that the main shoot apex had been damaged. In the present work, in which a considerable number of decapitated shoots, and short lengths of shoot, were planted out, only a few developed lateral buds (Pl. XXVII, Fig. 8) (as distinct from the terminal regenerations described below). In anatomical studies it was ascertained that these lateral buds are typically formed at or near the surface of the shoot in proximity to the point of conjunction of two vascular strands, i.e. at the 'closing' of a leaf-gap. The position of a lateral bud in *Ophioglossum* is thus closely comparable with that of a leptosporangiate fern, where the bud arises from a detached meristem (Wardlaw, 1943 *a*, *b*). In these buds the apical meristem was clearly apparent before the appearance of the first leaf primordium (Pl. XXX, Fig. 24).

In shoots which had been decapitated just below the apical meristem a surprisingly vigorous regeneration took place at the distal cut surface. The operational procedure consisted in observing the plant under a binocular microscope and in removing thin transverse slices until it was evident that the shoot apex had been excised. The regeneration thus took place in the soft immature tissue close to the apex. In the course of some 3–4 weeks a small, pale-green, mound-like outgrowth could be observed at the surface of the central pith, and after 5–6 months a large pale-green terminal leafy bud had been formed (Text-figs. 10, 11, and Pl. XXVII, Fig. 2). At an intermediate stage the new growth was massive and had a callus-like appearance; on further growth, distal and lateral protuberances indicated the positions of the first leaf and first root respectively (Pl. XXVII, Fig. 9). Where a single shoot had been regenerated it occupied a central or approximately central position in the shoot (Pl. XXVII, Fig. 2) and was scarcely distinguishable from a normal apical bud from which the current year's leaf had been removed. In some specimens, however, more than one bud was formed, the lateral buds having apparently originated from cortical tissue (Pl. XXVII, Fig. 1). In several specimens there was one large bud and a small one in which development had apparently been arrested at an early stage. In each of five plants which were kept in culture over the winter a small non-spikate leaf developed in spring. On being sectioned these materials yielded information of interest on the inception of endogenous buds and the rate of formation of new leaves.

The inception of buds in the decapitated sub-apical region may be briefly indicated as follows. The immature parenchymatous cells of the pith at and near the cut surface begin to elongate and divide actively by transverse walls, so that a cambiform layer is formed; a slight outgrowth of tissue therefore becomes evident to the naked eye (Text-fig. 10; Pl. XXX, Fig. 20). This

outgrowth increases in size both vertically and laterally. Meanwhile an ellipsoidal mass of deeply staining cells develops within this new growth (Pl. XXX, Fig. 20). These cells are initially equidimensional, densely protoplasmic, and in a state of active growth; they are, in fact, embryonic or meristematic cells. This ellipsoidal, embryonic tissue mass is alined longitudinally within the shoot. On further development a nascent shoot apex can be observed near the centre of the lower half of the ellipsoidal mass (Text-fig. 12; Pl. XXX, Fig. 21), in fact, near the focal point. The essential feature in the formation of the

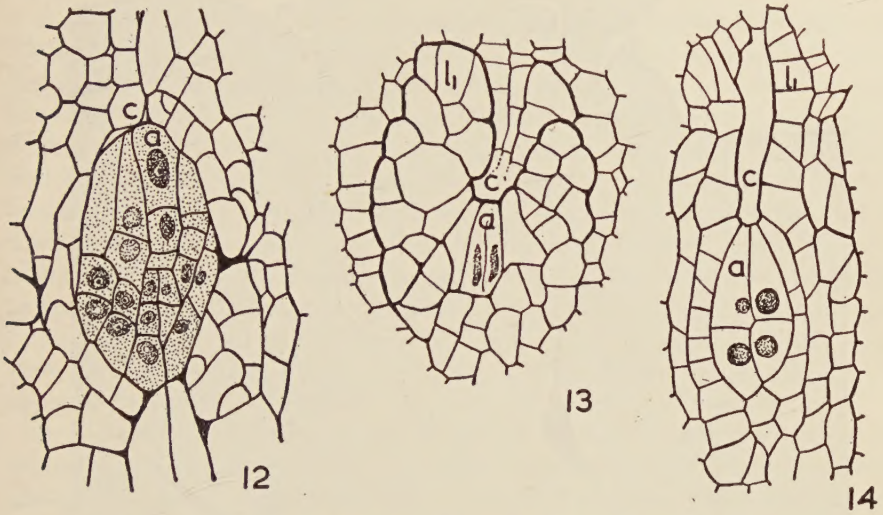


TEXT-FIGS. 10 and 11. Regenerative growth from the pith of a decapitated shoot. Fig. 10 shows an early stage, after a few weeks; Fig. 11 shows an older stage after 4–5 months. l_1, the first leaf of the endogenous bud; *s*, sheath, formed from pith parenchyma; *r*, roots of parent shoot. (× 4.)

apex is that the meristematic cells in the position specified become elongated and divide longitudinally, a group of parallel prism-shaped cells being thus constituted (Pl. XXX, Fig. 21). Simultaneously the walls of the cells immediately above and in contact with this nascent apex begin to undergo lysis, and this, together with the tensile stress generated by the enlarging and dividing prism-shaped cells, results in freeing the upper surface of the new apical meristem and in opening up a narrow longitudinal channel above its central point (Text-figs. 12, 14, and Pl. XXX, Figs. 22, 23). It can also be seen that the cells adjacent to the developing apex are typically dividing by walls at right angles to it (Pl. XXX, Fig. 21). In this we see the beginning of a concentric organization in the tissue surrounding the developing endogenous apex. At this stage, also, the outermost and uppermost cells of the outgrowth lose their cambiform arrangement and become distended parenchymatous cells (Pl. XXX, Fig. 20).

The formation of a rapidly growing leaf primordium on one flank of the apex takes place almost immediately (Pl. XXX, Figs. 22, 23; Text-figs. 13, 14).

This primordium forces its way into the overlying meristematic cells, a longitudinal channel being formed above its distal extremity. As in the normal development, outgrowths of cells near the basal margins of the new leaf take place: these give rise to the stipular or sheathing base. (Each such sheathing leaf-base completely encloses the shoot apex and all the younger leaf primordia. The green foliage leaf which appears each year is at first encased in



TEXT-FIG. 12. Inception of apex, *a*, of an endogenous bud, formed in the pith of a decapitated shoot. A group of cells within the ellipsoidal mass of meristematic cells has elongated and divided by longitudinal walls. The adjacent cells are dividing by anticlinal walls, and a lysis of the overlying cell walls, which will result in the development of a cavity, *c*, above the apex, is beginning to take place. (See Pl. XXX, Figs. 20, 21.)

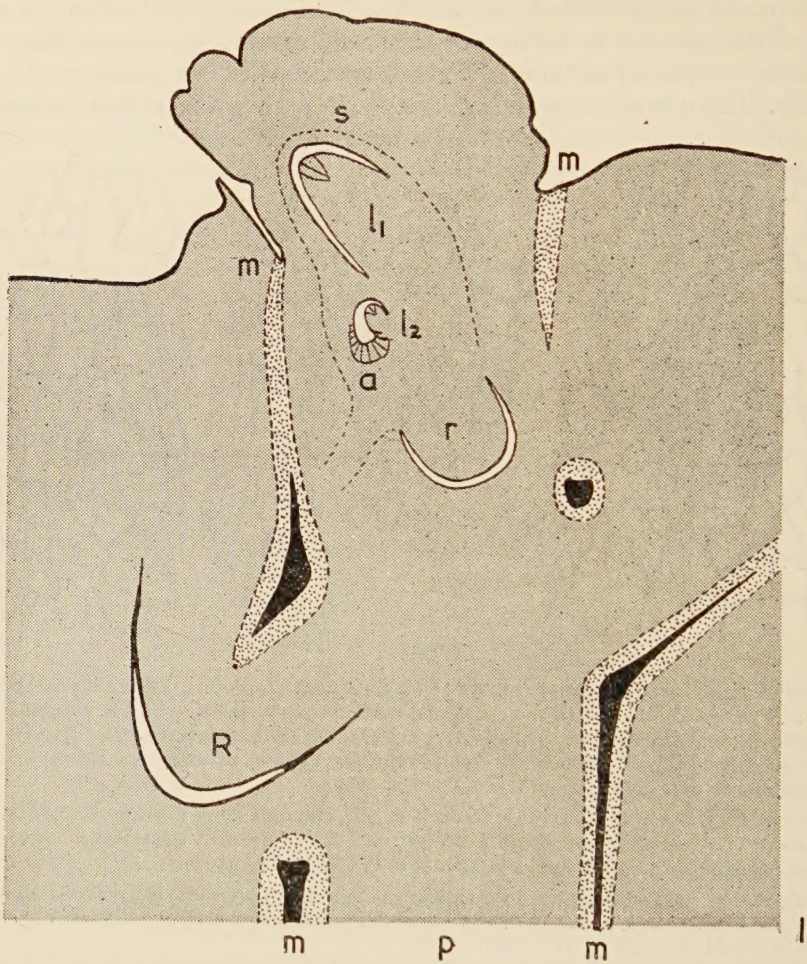
TEXT-FIG. 13. As in Fig. 12: the apical cell, *a*, is in a state of active division; the first leaf, l_1, consists of large cells, apparently in a very active state of growth; there is also an actively developing upgrowth on the opposite side. (See Pl. XXX, Fig. 22.)

TEXT-FIG. 14. As in Figs. 12 and 13. The apex of an endogenous bud in a decapitated shoot, showing the apical cell, *a*, the overlying cavity, *c*, and the first leaf, l_1 (not cut in median plane). (See Pl. XXX, Fig. 23.) (All × 225.)

the sheath of the leaf of the previous year. It has to thrust its way through this sheath before it can appear above ground.) Now, in these induced endogenous buds, there can be no 'normal' encasing leaf-sheath for the first leaf: nevertheless, such a sheath-like structure is present, the changes induced in the adjacent meristematic cells and surrounding pith parenchyma being such that they constitute a structure which is virtually identical with a normal sheath. The concentric cell layers which surround the developing leaf consist of deeply staining cells, almost of a tapetum-like character, as in the normal development.

The development of the first leaf in some of these endogenous buds presents some curious features. In several of them an organ, which appears to be the first leaf, consists of rather large distended cells evidently in a very

active state of growth (Pl. XXX, Fig. 22; Text-fig. 13). A similar but rather less developed lateral outgrowth of the meristem, approximately opposite the



TEXT-FIG. 15. Endogenous bud in the pith at the distal end of a decapitated shoot (semi-diagrammatic, based on three superimposed camera-lucida drawings). *m*, meristeles of parent shoot; *p*, pith; *R*, root of parent shoot; *a*, bud apex; l_1, l_2, leaf primordia; *r*, bud root; *s*, sheath of first leaf, consisting of modified pith parenchyma. The broken line round the bud indicates the outer limit of the deeply staining meristematic tissue and the incipient vascular strand joining the bud to a meristele. (× 55.)

first leaf, can also be seen in this specimen. In other endogenous buds, leaf formation has followed the normal course.

At an early stage, and usually in close relation to the base of the first leaf, a root initial can be observed; in due course this gives rise to a bulky root (Text-fig. 15). Below the bud there is a basipetal differentiation of vascular tissue, the vascular strand traversing the pith, often in an obliquely transverse direction, to become conjoined with one of the shoot meristeles.

These, then, are the several stages which characterize the remarkable phenomenon of the inception of an endogenous bud in the distal region of the pith. Taken together, the several developments which have been described raise many problems for discussion, including, in particular, the nature of the organizing properties exemplified by the apical meristem.

(*d*) *The effect of partial and complete defoliation*

In the normal development one leaf of *O. vulgatum* (with or without a fertile spike) appears above ground each year; occasionally two leaves appear, but this is considerably less common. In relation to the general thesis that the older leaves, or leaf primordia, regulate, or partially inhibit, the growth of the younger ones, the expanded leaf of the current year (as at mid-June) was removed by dissection round its base; the conical apical bud, with its encasing leaf-sheath, was thus disclosed. These plants and untreated controls were planted at the soil surface so that new foliar developments could be observed directly. No leaves were expanded in any of the treated plants between June and December. As already mentioned, the untreated controls retained their leaves until early November. Of twelve treated plants which were kept in pot culture over the winter, all produced one small, non-spikate, expanded leaf, with a somewhat circular lamina, the following April.

In materials of *O. vulgatum* obtained from the same source as before at the end of February, it was seen that the enlargement of the lamina and the elongation of the petiole had already begun. This leaf was removed from each of four stout specimens: after 3 months there was no evidence of a new leaf being expanded either in plants at the soil surface or below ground, but after a further 4–5 months small leaves, about 1·0–1·5 cm. long, had appeared.

In another defoliation experiment, in which large plants with stout rootstocks were used, all the leaves including the youngest leaf primordia were removed. (The possibility of a very small, recently formed primordium being left cannot be completely excluded.) In an initial experiment three such shoots were planted at the surface of moist soil under a bell-jar, the exposed apices being at first protected by small pieces of moist cotton-wool. All three apices survived the treatment, grew rapidly, and in the course of 4–5 months each had formed a large terminal leafy bud. These resembled the terminal bud in normal plants and were commensurate with, if not slightly larger than, those obtained by regeneration following decapitation. The single specimen which was kept in pot culture over the winter produced a small, non-spikate leaf the following spring.

Discussion

Holm (1925) has enumerated the plants in which shoot-bearing roots are known to occur and has cited the relevant literature. The interest of these shoot-bearing roots is manifold. Here we are specially concerned with the actual morphogenetic process and particularly with the very curious fact that the induced buds in *Ophioglossum*, whether in the root or decapitated shoot,

are endogenous and are formed from unspecialized parenchymatous tissue. This is in marked contrast to other ferns, such as *Matteuccia*, *Onoclea*, *Dryopteris*, &c., where the lateral shoot buds always have their inception in superficial, detached meristems which originally formed part of the apical meristem, i.e. in these ferns the histological basis of a bud meristem already exists (Wardlaw, 1943*a*, *b*).

From the morphogenetic standpoint the principal problem is then to explain how some particular effect, e.g. that of a 'bud-forming substance', is induced in a particular meristematic cell so that it becomes a locus or centre of growth, giving rise to a bud on further development. As a correlative development, the growth of the adjacent main root apex is greatly diminished, if it is not completely inhibited. In *Ophioglossum vulgatum* lateral rootlets are completely absent: hence the hypothesis that endogenous buds are modified lateral roots is ruled out (see Goebel, 1902), as it is by the evidence both of Rostowzew and that now presented. Where the bud originates in the middle cortex at some point along the root we have to consider how it happens that a particular group of parenchyma cells becomes a locus of growth or growth centre.

Goebel (1902) has suggested that a 'bud-forming substance' is normally present in roots and may be translocated into the shoot; and since a detached root already contains this substance, it can give rise to a bud or buds. Again, since buds are formed much more quickly on detached roots with tips than on detached decapitated roots, and since buds are usually formed at the tip, it would appear that this substance is either formed at the root apex, or that it tends to accumulate there. The accumulation of the bud-forming substance at some point along the root is more difficult to explain: this, indeed, is a problem not unlike that of the formation of endogenous lateral rootlets at some points in a root but not at others. The localization of the bud-forming substance is affected by gravity, the buds being always formed in the upper half of the pegged-down isolated roots. In due course the young bud shows polarity: it is typically alined in the vertical axis of the ellipsoidal meristematic mass of tissue.

In exogenous buds it is usually the most distal group of meristematic cells of the regenerative outgrowth that constitutes the new apical meristem. From the normal stem apex there is support for the view that the apical cell group in vascular plants in general has, by virtue of its unique position in the shoot, a special and distinctive metabolism. It is a growth centre, occupying, as it were, a peak of metabolic potential. In the distinctive metabolism of the apical cell group there may be some substance (or combination of substances) which has the effect of keeping these cells in an active, highly embryonic condition. Such a substance is known to be present in coco-nut milk, and the same or similar substances may be present in all embryonic cells. In the organization of the endogenous shoot apex in the homogeneous ellipsoidal meristematic mass in *Ophioglossum* there appear to be two separate though probably related problems: one is to account for the fact that it is a central

group of cells that acquires special metabolic properties; the other is to account for the particular kind of organization, i.e. that of a shoot apex, actually developed. It seems necessary, in general terms, to postulate that a biochemical or metabolic pattern precedes and underlies the visible histological pattern; and this biochemical pattern will be determined in conformity with the laws of physical chemistry as they apply to a specific organic reaction system. Certainly, in *Ophioglossum*, there must be some distinctive feature, possibly as Goebel postulates a substance, which organizes the embryonic activity in such a way that a stem apex, and not, for example, a root apex, is produced. The nature of this is, of course, one of the major phenomena that await explanation. It may be that the inception of the stem apex takes place at a critical partial pressure of oxygen or of carbon dioxide, and that the required condition is only realized at a certain position within the meristematic mass. The endogenous origin of buds and the fact that the normal apex is completely enclosed within several layers of sheathing leaf-bases make it not improbable that gaseous composition may be a factor in the inception and maintenance of the apical meristem. White (1939), on the basis of studies of surface and submerged *Nicotiana* tissue cultures, has suggested that low oxygen tension promotes bud formation. Wardlaw and Allsopp (1948), on the other hand, found that the formation of buds in various ferns was unaffected when the partial pressures of oxygen in nitrogen were widely varied.

It is now generally accepted that the shoot apex in vascular plants is a self-regulating, organizing, or morphogenetic region. This is equally true of the endogenous apex of *Ophioglossum*. Moreover, its organizing activity is not confined to the organs and tissues directly formed from it but it extends to the circumambient tissue also. This organizing activity is evident from a very early stage: it is seen in the lysis of the cell walls and the formation of an air space above the nascent apex, and in the formation from parenchyma cells of a characteristic sheath round the bud. From such observations it is clear that a close study of endogenous bud apices is desirable, if only to extend our conception of the potentialities of apical meristems in general.

In respect of their vascular development, the endogenous buds of *Ophioglossum* closely resemble other ferns. They afford evidence that as soon as the shoot and leaf apices are formed there is a basipetal movement of some substance which induces rapid cell division; the incipient vascular strand which is thus formed extends back to the stele in the case of root-buds; i.e. there is evidence that gradients or tensions have been set up between the bud apex and the root stele.

Campbell (1911, 1940) has recorded that in very young embryos of *Ophioglossum* species the shoot apex is very difficult to discern; indeed, he has suggested that initially it is not present at all, the first apex being that of the first leaf, and several leaf apices being formed before a shoot apex becomes sufficiently organized to be clearly distinguished. The leaves, however, form a normal phyllotactic sequence round a central focal point—the non-apparent

shoot apex. That a small, though histologically insignificant, apex is present, however, can scarcely be doubted. Bruchmann (1904), in an account of the embryogeny in *O. vulgatum*, states that a root and foot are first formed and then one or more leaves. The present study of the inception of endogenous buds has shown beyond any doubt that a shoot apex is first organized and that the first leaf, though it appears very soon after the apex, is formed in a normal relation to it; and so on for the second and third leaves. The organization of the new leafy shoot, in fact, is determined by the shoot apex. For the present it must suffice to note that the nutritional relationships of the embryo, borne on the prothallus, and of buds formed on well developed shoots or roots, are likely to be very different indeed, and, as recent investigations by Allsopp (1952, 1953), Wetmore *et al.* (1949, 1950, 1951), and Wardlaw (1952) have shown, the nutritional status of the apex is important in determining its morphogenetic expression.

Summary

Root-buds are known in several species of *Ophioglossum* and are the common means of propagation. In an experimental investigation of bud formation in *Ophioglossum vulgatum* root-buds have been induced in both entire and decapitated isolated roots. While some buds may be formed from a segment of the root apical meristem, as described by Rostowzew, those which occur at random along the root develop from parenchyma cells of the middle cortex and are endogenous. Endogenous buds have also been induced in the pith parenchyma of shoots from which the apex had been excised. In the formation of these endogenous root- and shoot-buds the following developments have been observed: the inception of a growth centre in a group of parenchyma cells; the rapid division of one or more of these cells to form a spherical or ellipsoidal meristematic cell mass; the formation of an upwardly directed nascent shoot apex by the elongation and longitudinal division of a group of cells within this mass; the lysis and distension of the adjacent cells above, so that the upper surface of the nascent apical meristem becomes freely exposed to the small air space thus formed; the early formation of a rapidly growing first leaf and first root; a concentric organization of the meristematic and parenchyma cells surrounding the apex so that they constitute a protective sheath which resembles a normal stipular sheath; and the basipetal development of an incipient vascular strand by the longitudinal division of the parenchyma cells situated below the bud, phloem and xylem being subsequently differentiated in this strand. Root-buds are not formed by the transformation of incipient lateral roots—these, in fact, do not occur in *O. vulgatum* —and they bear no obligate relationship to the orientation of the root stele. The subsequent developments leading to the formation of a young plant, with a shoot apex and several leaves and roots, are as in the normal development.

These bud developments are very different to those observed in the embryogeny. In the young sporophyte the first distinguishable organs are the foot and the root, the latter being well developed before any apical bud can

be discerned; moreover, the first leaf is rudimentary while the second is said to take several years before it appears above ground. These differences between bud formation on roots and shoots and the development of the sporophyte on the prothallus illustrate the importance of nutritional factors in morphogenesis. In the inception of buds, a nascent shoot apex is the first organ to become apparent and, like other shoot apices, it functions as a self-determining, organizing region, its effects extending into the surrounding parenchymatous tissue in which the endogenous bud is enclosed.

No adequate explanations can yet be given of the successive stages in the inception and development of these induced endogenous buds, but an attempt is made to indicate what the relevant problems are.

The author has pleasure in thanking Mr. E. Ashby for his help in microscope preparations and photographic illustrations.

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EXPLANATION OF PLATES

Illustrating Professor C. W. Wardlaw's paper on 'Experimental and Analytical Studies of Pteridophytes. XXIII. The Induction of Buds in *Ophioglossum vulgatum* L.'

(All figures are from untouched photographs)

PLATE XXVII

Fig. 1. Formation of three buds at the upper surface of a shoot from which the apex had been excised. (×7·5.)

Fig. 2. Formation of a bud, as in Fig. 1. (× 5.)

Fig. 3. A small endogenous bud bursting out from the root cortex. (× 5.)

Fig. 4. A typical root-bud, formed at the root-tip, and a second, smaller bud, also formed at the root-tip. (× 5.)

Fig. 5. Two examples of typical root-tip buds. (× 5.)

Fig. 6. Two endogenous buds formed in close proximity in the mature region of a root. (× 5.)

Fig. 7. Three buds formed together in the mature region of a root. Note the extensive hypertrophy of the root tissue. (× 15.)

Fig. 8. A bud formed laterally on a piece of decapitated shoot. (× 5.)

Fig. 9. An early stage in the formation of a bud at the distal end of a decapitated shoot: the bulges indicate the positions of the first leaf and the first root. (× 5.)

PLATE XXVIII

Fig. 10. The earliest visible stage in the inception of an endogenous bud in the mature region of a root. Cells of the middle cortex have begun to divide. The edge of the root stele can be seen below. (× 225.)

Fig. 11. As in Fig. 10, but a little later. Very active division has taken place in one of the dividing cells. (× 225.)

Fig. 12. As in Figs. 10 and 11. The actively dividing cell has given rise to an ellipsoidal mass of deeply staining, small meristematic cells. (× 225.)

Fig. 13. The shoot apex of an endogenous bud subsequently formed in an ellipsoidal tissue mass. (× 225.)

Fig. 14. The shoot apex and first leaf primordium of an endogenous root-bud. (× 225.)

Fig. 15. The basal region of an endogenous bud as seen in a transverse section of the root. As a result of a basipetal stimulus from the leaf and shoot apices, the cells of the inner root cortex have divided actively to form an incipient vascular strand. Cells in proximity have also been stimulated to divide. The edge of the root stele can be seen at the bottom of the photograph. (× 100.)

PLATE XXIX

Fig. 16. An endogenous bud in longitudinal section, showing the first leaf and its stipular base and the shoot apex. (× 225.)

Figs. 17 and 18. Mature region of roots in transverse section, showing early stages in the formation of endogenous buds from the tissues of the cortex. It can be seen that some of the outer cortical tissue has become transformed into a sheathing tissue surrounding the developing bud apex and first leaf. (× 40.)

Fig. 19. Longitudinal section of a root apex showing a terminal bud with its deeply seated apical meristem, its first leaf, which has burst through a leaf-sheath organized out of cortical tissue, and the downwardly deflected tip of the parental root. (× 25.)

PLATE XXX

Fig. 20. Longitudinal section of the pith in the distal region of a decapitated shoot, showing regenerative growth leading to the formation of an endogenous bud. In the centre of the

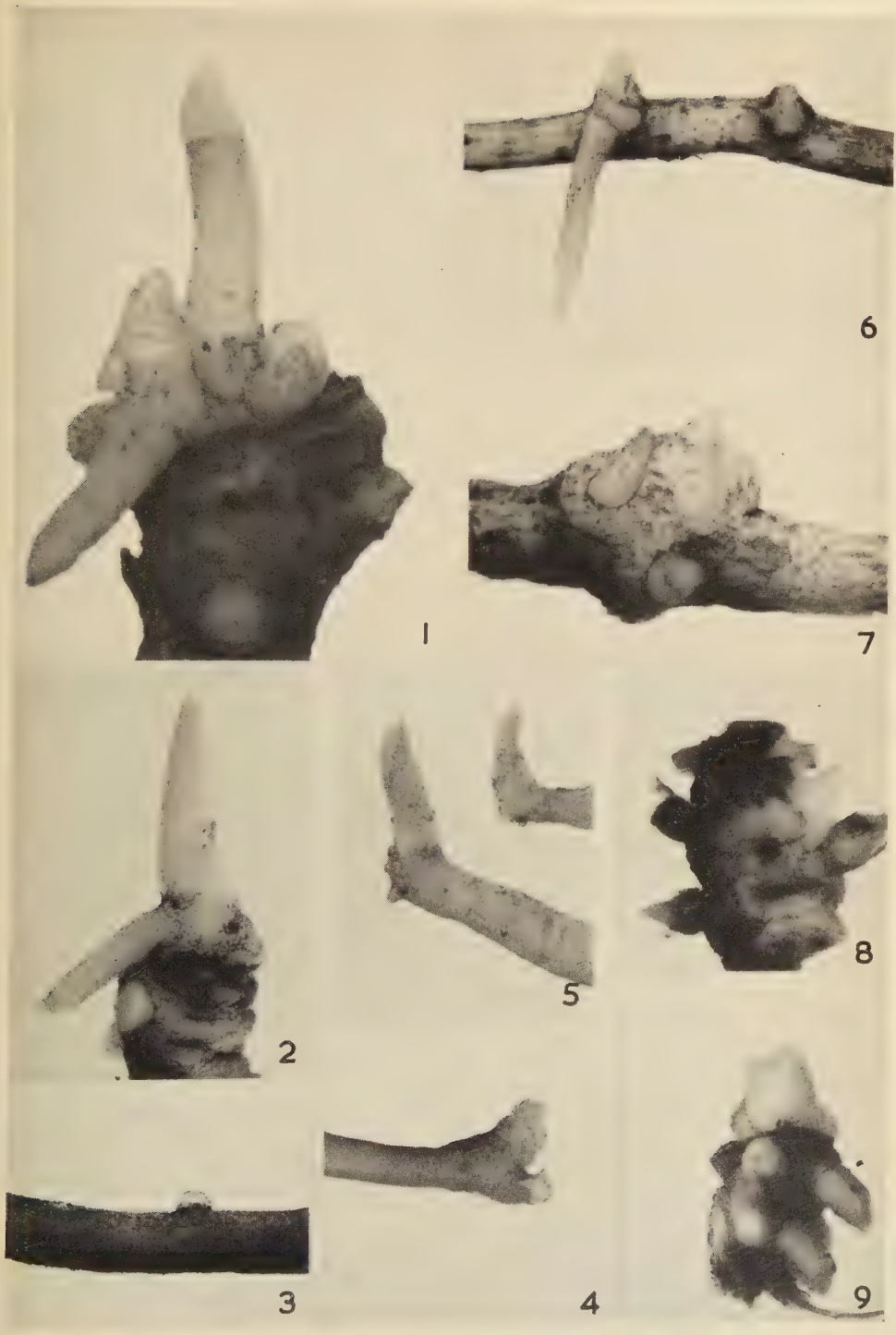
mound-like upgrowth there is a densely staining ellipsoidal mass of meristematic cells within which the nascent shoot apex can be seen. (See also Fig. 21.) (× 80.)

FIG. 21. Part of Fig. 20, showing the nascent bud apex. Some of the meristematic cells have elongated and divided by longitudinal walls. The surrounding cells are being thrust outwards and are dividing by walls which are anticlinal to the new apex. (× 225.)

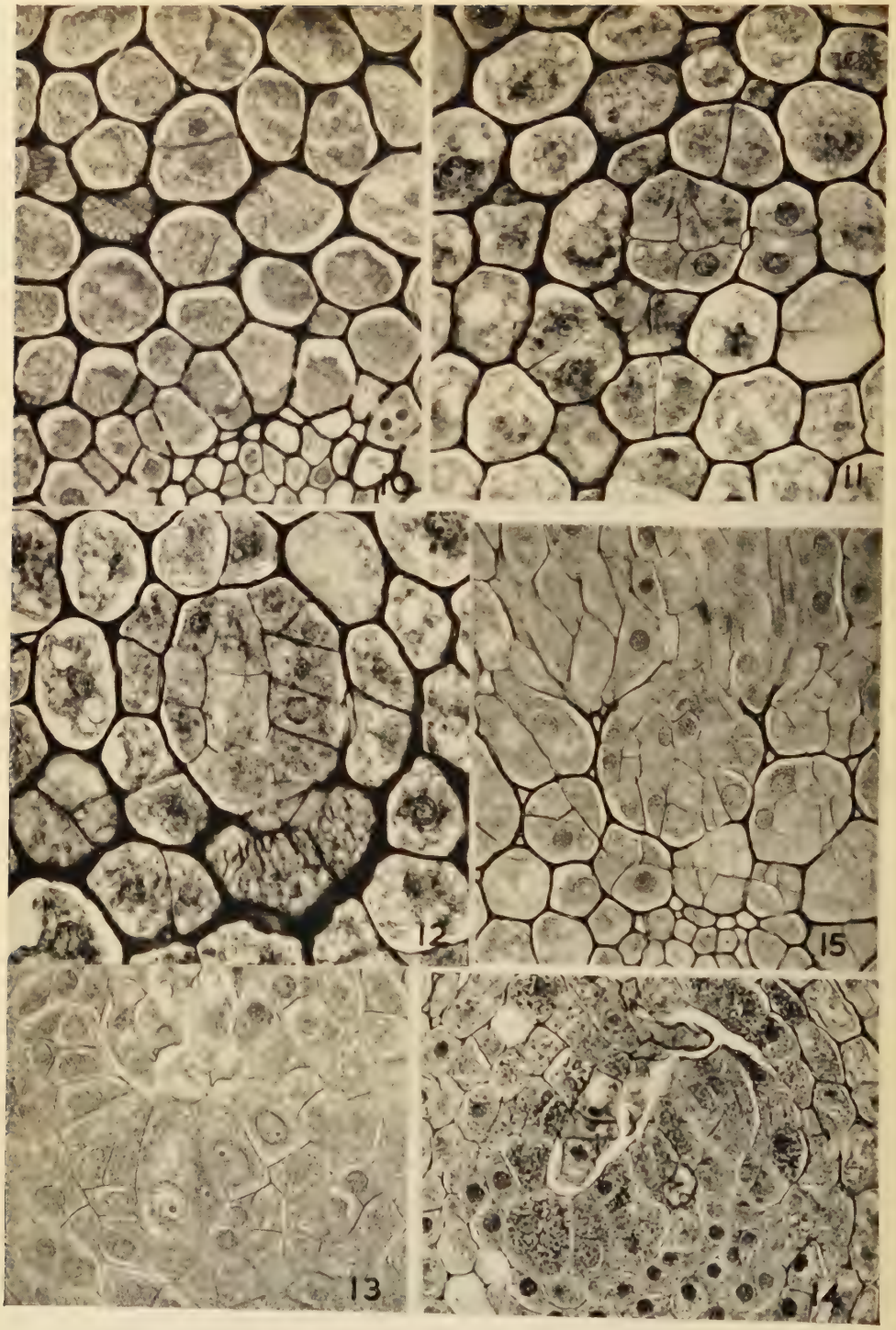
FIG. 22. The apical region of an induced endogenous bud, as in Figs. 20 and 21, showing the shoot apex, *a*, and a very rapidly growing leaf on the left, and a similar but smaller upgrowth on the right. (× 225.)

FIG. 23. The apical region of an induced bud as in Figs. 20–22, showing the dividing apical cell, *a*, the cavity above the apical meristem containing mucilaginous hairs, and the first leaf primordium (on the right). (× 225.)

FIG. 24. The apex of a lateral shoot bud, showing the apical meristem prior to the formation of the first leaf. Some mucilaginous hairs are present in the cavity above the meristem. (× 225.)



C. W. WARDLAW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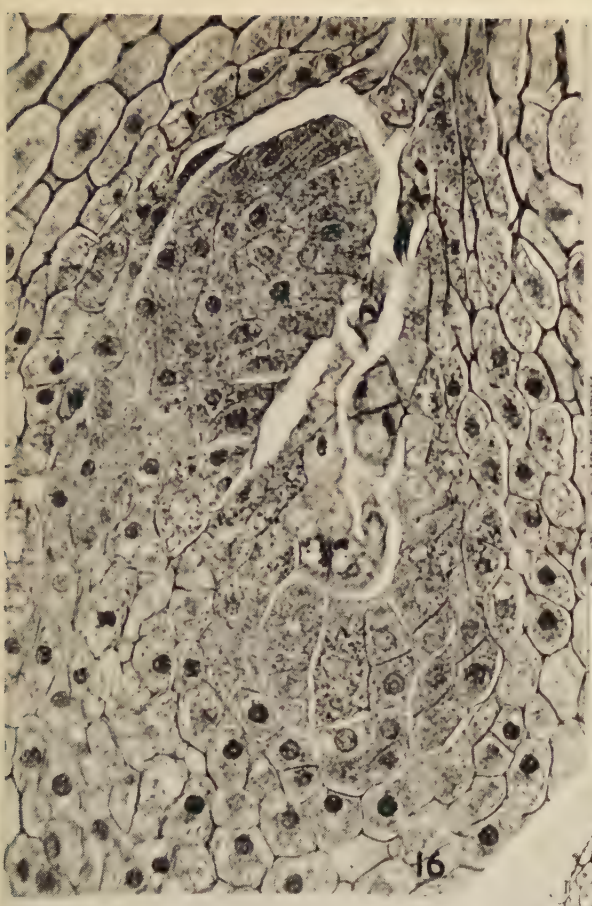

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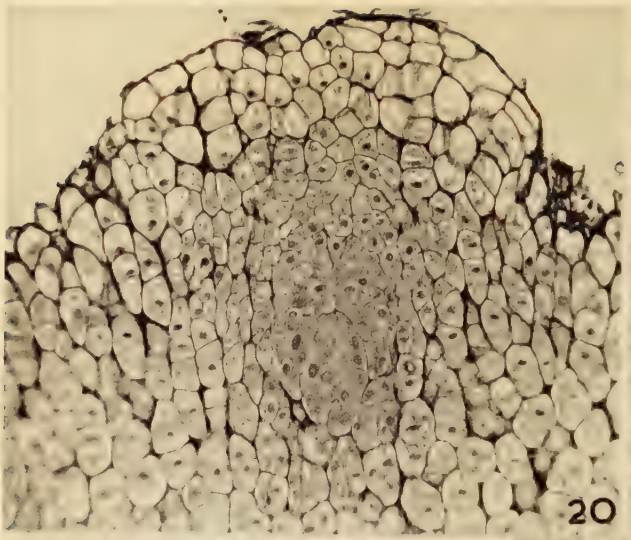
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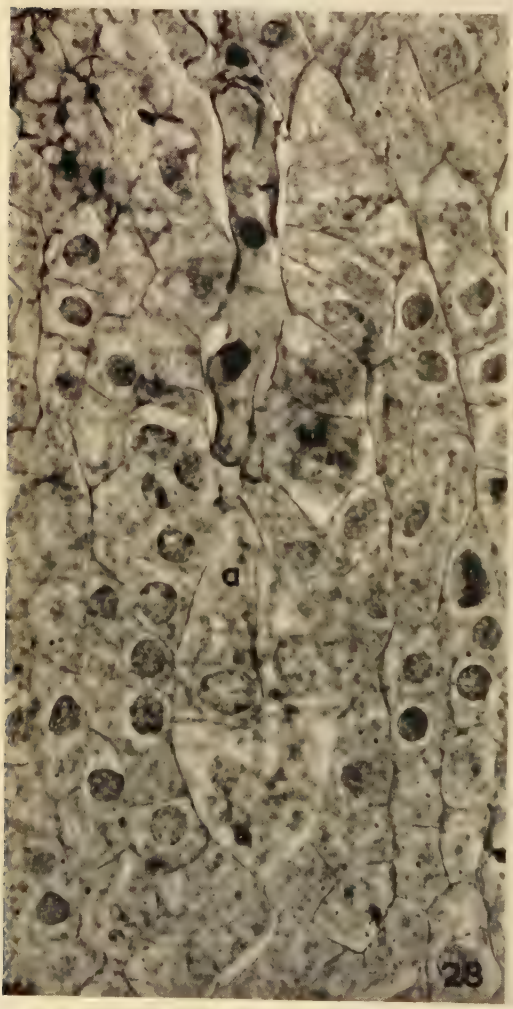
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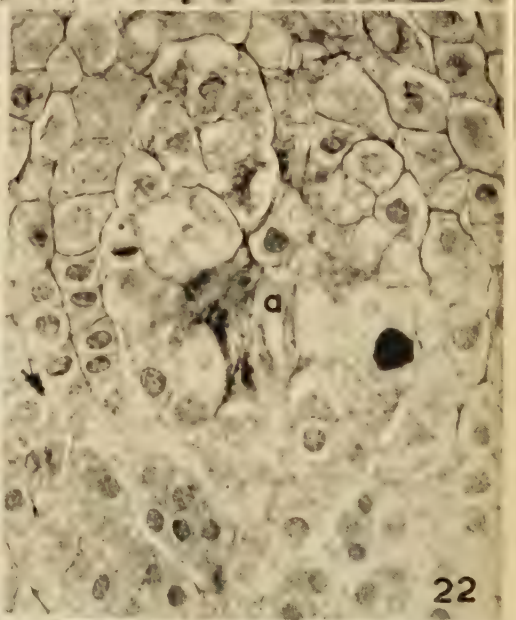
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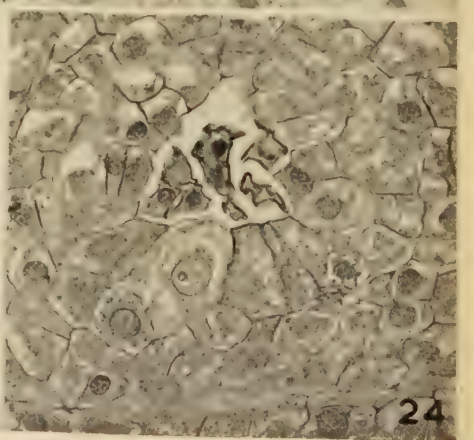
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Physiological and Ecological Studies in the Analysis of Plant Environment

VIII. An Inexpensive Integrating Recorder for the Measurement of Daylight

BY

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With two Figures in the Text

Abstract

The design is based on the principle that changes in the current passing through a vacuum emission cell, induced by variations in the light quanta received, control the rate of charge of a condenser. When the striking voltage of a discharge tube is reached the resultant flow of current energizes an electromagnetic relay which, in turn, is connected with a Post Office counter. In order to eliminate possible errors due to changes in temperature, the compartment containing the discharge tube is kept at a constant high temperature (100° F.), while a stabilized mains source of electricity is used. A ground-glass diffusing dome is used and a filter to exclude infra-red and ultra-violet radiation. With a photometric cell of relatively uniform sensitivity in the visible spectrum the recorder gave a linear response up to the maximum intensity investigated. No change in the calibration factor occurred in two summers' continuous use in the open. The electrical components, including the photo-electric cell, cost *c.* £25, while the construction only demands normal workshop facilities.

Introduction

IN a previous paper (Blackman and Wilson, 1951) evidence was produced that the seasonal change in the net assimilation rate of *Helianthus annuus* is closely related to the changes in the total light received per day. In order to investigate this relationship more closely, an accurate instrument was required to integrate the light received per day over the wave-lengths important in photosynthesis. Since no instrument was commercially available in Great Britain, the present recorder was designed and constructed.

Two basic principles have been employed for the integration and recording of light intensity and radiant energy. In one, the radiant energy raises the temperature of a blackened thermocouple and the resultant current is

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measured by a suitable recording galvanometer. In the other, light falling on a photo-electric cell either generates a current or causes changes in the current flowing through the cell.

Instruments of the thermocouple type have been used frequently in meteorological work, and Kimball (1927, 1930) lists the types then in operation at stations throughout the world. A review of the results obtained in the United States has recently been published by Hand (1949). In addition, instruments of this type have been employed in ecological and physiological studies by a few workers, for example by Gast (1930) and by Watson (1947).

From the viewpoint of investigations relating to the assessment of light as an environmental factor, thermo-electric instruments have various disadvantages. Unless appropriate light-filters are fitted, the records for total radiant energy will include the energy derived from infra-red and long red wave-lengths which are not concerned in photosynthesis, and most of the standard instruments do not appear to have been fitted with such filters. Furthermore, such instruments measure the energy while the response of photo-electric cells is linked with the quanta received. It is now widely held (e.g. Nishimura, Whittingham, and Emerson, 1951) that the photosynthetic process requires the absorption of a certain number of quanta per molecule of oxygen produced; hence it is the number of quanta reaching the leaf rather than their energy which needs to be measured. Therefore in investigations of this type a photo-electric instrument in which the response is determined by the number of quanta is preferable.

There are two types of photo-electric cell which have been used in light-recording instruments. Firstly there is the 'barrier-layer' cell, in which the absorption of light brings about the generation of a small electric current, and which demands no external source of current for its operation. This type of cell has the disadvantage that except over relatively narrow ranges of light intensity the current generated is not proportional to the light intensity received and, in consequence, when such cells are used to integrate the diurnal changes in light intensity considerable errors may be introduced. Secondly there is the 'emission' cell which from the aspect of a linear response can be much more satisfactory. Such cells require an external source of current for their operation and it is necessary to operate the cell above the saturation voltage when the current is proportionate to the intensity.

A recording light-meter incorporating an emission cell appears to have been used first by Ives (1925), who coupled a Case aluminium–barium oxide cell to a Leeds and Northrup recording galvanometer. This cell has a maximum sensitivity in the blue-violet, but to overcome this defect the light was passed through a yellow filter. The instrument was used for recording seasonal changes of light intensity in the open, but since Ives himself questioned the methods of calibration, these records are of limited value.

Atkins and Poole (1930, 1936) described a similar instrument employing a Burt sodium vacuum cell and a Cambridge thread-recorder. This cell also has maximum sensitivity in the blue end of the visible spectrum, but no

filter seems to have been added. The instrument, set up on the roof of the Marine Biological Laboratories at Plymouth, was in operation continuously for some years, and records of the seasonal and diurnal changes in light intensity have been published by Atkins and Poole (1936) and by Atkins and Ellison (1947). A similar instrument, employing a Weston copper–copper oxide emission photocell and a recording galvanometer, was described by Maclean (1940).

Rentschler (1930) constructed a light-meter for the measurement of ultra-violet light. In his design there was no direct measurement of the current on a recording galvanometer, but changes in the resistance characteristic of the photocell controlled the current passing in the circuit and thereby the time taken to charge a condenser. When the voltage across the condenser reached the striking voltage of a discharge tube, the resultant flow of current energized an electromagnetic relay, which in turn operated a telephone call-meter, while at the same time the condenser discharged and commenced recharging. By the use of a light source of known intensity the number of discharges recorded on the meter per unit time could be determined. An instrument aiming at the accurate measurement of light quanta and based on this circuit has also been described by Muller and Shriver (1935), who discussed its performance and limitations in considerable detail.

Sprague and Williams (1941, 1943) also employed the same basic circuit using a General Electric PJ22 photocell. For a counting mechanism they converted an alarm clock by removing the balance-wheel and connecting the escapement directly to the armature of the relay by a fine spring wire. The number of discharges was thus recorded by the position of the hands of the clock. By minor modifications of the circuit the instrument could be operated in the field from batteries or in the vicinity of the laboratory from the main electricity supply.

More recently Somers and Hamner (1951) have described a recorder based on the design of Sprague and Williams but incorporating a series of eight counters and a commutator so that the total amount of light per day could be obtained for a period of a week. Using a Radio Corporation of America photo-tube 922 together with two Corning filters, the relative sensitivity varied between 100 at 6,000 Å to *circa* 40 at 4,400 Å with a secondary peak of *circa* 70 at 4,000 Å. With a Weston Sunlight Illumination Meter (No. 603) as the standard, the response was linear up to 9,000 foot-candles, while in comparison over an extended period with an Eppley pyrheliometer a high correlation was obtained between the two instruments.

Design and Construction

The well-known principle was adopted of using a circuit in which the flow of current through the emission photo-electric cell controls the rate of charging of a condenser, and thus the number of discharges made by a discharge tube. The circuit described by Rentschler (1930) and the modified design for field use put forward by Sprague and Williams (1941, 1943) were selected

as being the most suitable. As this system incorporates a counting-mechanism to record the number of discharges, it is possible to obtain a direct reading for the integrated light intensity over a convenient period, without having to measure the area enclosed by the curve traced by a recording galvanometer. The substitution of a counter has the advantage not only of simplicity but also of accuracy, since in periods of rapidly fluctuating light intensity, such as frequently occur when the sky is covered with small scattered clouds, a graphical record becomes increasingly difficult to interpret.

The choice of a suitable photo-electric cell is clearly of primary importance in the design of an instrument of this type, and consideration was given to the following criteria: (*a*) In any type of integrating instrument the accuracy is dependent on the response being directly linked with the quanta received by the cell. (*b*) Previous workers have in nearly every case used a cell with maximum sensitivity in the blue regions of the spectrum, but a cell with uniform sensitivity over the range of wave-lengths significant in photosynthesis was considered preferable. (*c*) It is highly desirable that the performance of the instrument should be independent of external factors, the most important of which is temperature. (*d*) There should be no drift in performance of the cell with time or, if this is present, it should be a progressive but steady change so that the drift in performance can be followed and the calibration factor altered accordingly. On the other hand, greater confidence can be placed on the records obtained if there is a constant response. (*e*) No current should flow through the cell in the absence of light.

After an extensive examination of the specifications of photo-electric cells available commercially, it was decided that the Cinema Television Ltd. photo-electric cell VB39 met the necessary requirements. It is a vacuum cell with which is associated good stability. It is also designed for accurate photometric work, with a projected cathode area of 10 sq. cm., while the cathode is completely surrounded by a cylindrical gauze anode for maximum collection of electrons, thus ensuring linearity of response. In addition, this photometric cell had the most uniform sensitivity in the visible spectrum. Thus, according to the maker's specification, while the maximum sensitivity is at 4,600 Å, the relative sensitivities at 4,000, 5,200, 5,800, and 6,400 Å are 0·85, 0·90, 0·67, and 0·40 respectively. Drift of performance with time and lack of current output in the dark were investigated during the operation of the instrument and will be discussed later.

The other two components which required careful choice were the counting device and the discharge tube. The counter decided upon was a standard Post Office call-meter. The discharge tube chosen was a Ferranti K3 cold cathode relay tube, the operating characteristics of which were considered to be the most suitable. Since the tube is gas-filled, its performance may vary with temperature, and it was therefore decided to incorporate in the instrument a thermostatically controlled heating system to maintain a constant temperature.

A diagram of the circuit developed is shown in Fig. 1. 230-volt A.C. mains

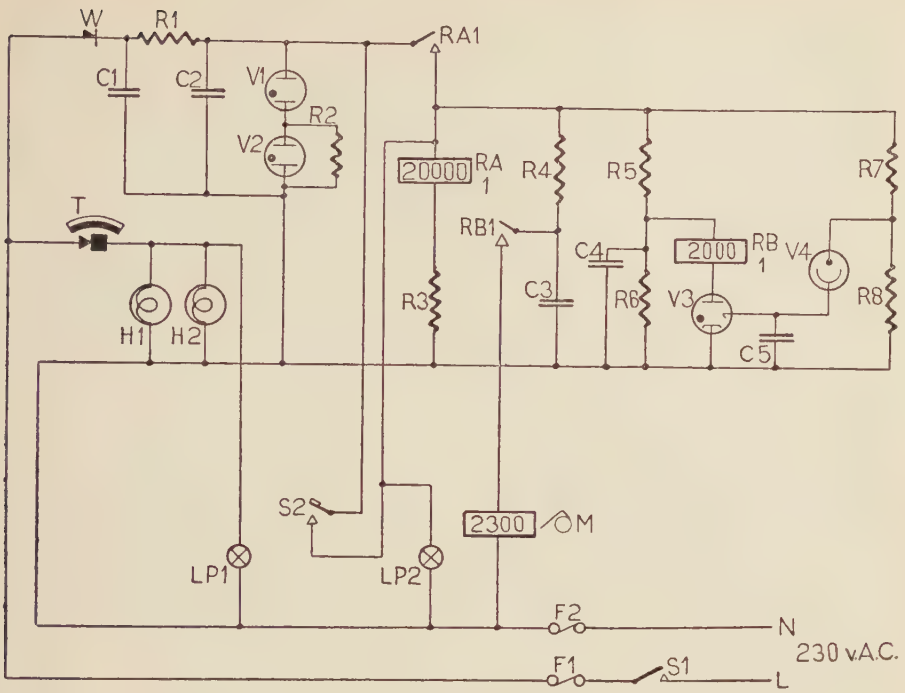

FIG. 1. Key to circuit diagram of light recorder:

Resistances (ohms)

R1	12 K	1 watt	
R2	1 M	¼	,,
R3	75 K	1	,,
R4	270 K	¼	,,
R5	270 K	¼	,,
R6	680 K	¼	,,
R7	75 K	½	,, (high stability)
R8	95 K	½	,, (,, ,,)

Indicator lamps

LP1 230 V. ½-watt neon
LP2 Neon indicator (to operate from 170 V.), actually CV1070 stabilizer in series with 270 K resistance.

W = Selenium rectifier 230 V. 5 mA.
T = Thermostat ½ amp. A.C.
M = Post Office call-meter (Type 100*c*) 2,300 ohms.

Condensers

C1 2 μF 350 V. D.C.
C2 ,, ,,
C3 ,, ,,
C4 ,, ,,
C5 2 μF 1,000 V. D.C. (Aerovox 1090)

Valves

V1 85A1 (Mullard) stabilizer
V2 ,, ,,
V3 K3 (Ferranti) gas triode
V4 VB39 (Cintel) vacuum photocell

Relays

Post Office Type 3,000 relays
RA 20,000 ohms 1 make
RB 2,000 ohms 1 make

H1 & H2 = Heaters—230 V. 15 watt pygmy sign-lamps
S1 = On/off switch
S2 = Push button switch
F1 & F2 = Fuses—250 mA.

supply was used as source of current, converted to D.C. by a metal rectifier, and stabilized at 170 volts by two Mullard 85A1 stabilizers (V1 and V2). The principle of the circuit has been described already: the current flowing through the photo-electric cell (V4) charges a condenser (C5), until the voltage across it reaches the striking voltage of the triode (V3). The resultant flow of current through the triode energizes the electromagnetic relay RB, which allows the current to flow to the P.O. meter (component M) and operate the counting-mechanisms. In order to avoid faulty readings arising from power cuts, a relay RA was added to the circuit in such a way that the circuit, once broken, had to be reset by hand, and a push-button contact (component S2) was provided for this purpose. It was intended to use a miniature neon indicator lamp to show the state of the circuit, but since at the time one to operate from 170 volts was not available, a CV1070 stabilizer tube (LP2) was used in its place.

Because of the possible effect of temperature on the discharge tube, it was necessary to add a heating system to keep the tube at a constant temperature. The simplest system appeared to be to maintain in the instrument an internal temperature higher than that encountered in the open at any time in the year. To do this two pygmy 230 volts 15-watt bulbs (H1 and 2) with a small bimetal strip 0·5 amp. A.C. blanket type thermostat (component T), set at 100° F. were incorporated. A neon indicator lamp (LP1) was added to show whether the heater bulbs were in operation.

The components were housed in two units; one, consisting of counter, indicator lamps, push button, and a mains switch, was placed in a greenhouse, and the other, containing all the other components, was mounted on the top of a standard Meteorological Office Stevenson screen in the middle of the experimental area.

The 'outside' unit consisted of two sheet-metal boxes, one inside the other, separated by a quarter of an inch of lagging felt to minimize temperature fluctuations within the inner box. The electrical components were mounted on a chassis, with the photo-electric cell in a light-proof partition to prevent light from the heater bulbs being measured as 'daylight'. In the lid of the inner box, immediately above the photo-electric cell, which was mounted horizontally, was a hole 4·5 in. in diameter, and a corresponding hole was cut in the lid of the outer box. A watertight 'porthole' mounting on the outer box held in position across the hole a disk of Chance ON 20 heat-resisting, heat-absorbing glass, inserted to prevent infra-red and ultra-violet radiation reaching the photo-electric cell. In order (*a*) to keep the light falling on the photo-electric cell within the makers' specifications, and (*b*) to prevent the meter failing to register counts, as occurred when the rate of discharge was more than about once a second, a piece of perforated zinc of 12 per cent. transmission was placed across the opening of the inner box. A diffusing hemispherical dome was made from an ordinary glass crystallizing dish, with a ground glass surface produced by hand with carborundum paste. The dome was held in position above the aperture by three rubber-lined spring toolclips.

Operation and Performance

The instrument was completed in February 1950 and in connexion with physiological investigations to be reported later was run continuously recording the light received per day between March 5 and October 20, 1950, and again in 1951 between April 1 and September 3.

Before use the instrument was calibrated, and subsequent calibrations were carried out at approximately 6-weekly intervals during the operational periods. Calibrations were made against a 250-watt substandard tungsten filament lamp operated at a constant voltage and previously calibrated in foot-candles by the National Physical Laboratory, Teddington. Calibration was made usually with the diffusing dome, Chance glass, and 12 per cent. perforated zinc screen in position, with the lamp at a distance of 1 ft. from the photo-electric cell, over periods of 1 hour. In the initial calibration in March 1950, and again in February 1951, longer periods were employed so as to obtain the most precise results. In addition, the figure was checked by removing the perforated zinc screen and recalibrating after making allowance for the changed transmission factor. From these calibrations—see Table I—it is evident that there was no appreciable drift in the performance of the cell and the calibration factor 1 discharge = 2·40 foot-candle hours was therefore used throughout the investigation.

Table I

The Variation in the Calibration Factor during the Operational Periods in 1950 and 1951

Date.	Quantity of light per discharge (foot-candles × hour).
1950	
23/3/50	2·40
3/5/50	2·41
21/6/50	2·43
6/8/50	2·38
18/9/50	2·41
1951	
2/2/51	2·40
21/5/51	2·42
9/7/51	2·39
13/8/51	2·39

Whenever the instrument was brought into the laboratory for calibration a check was also made to confirm that no current flowed when the cell was in darkness, and on each occasion a negative result was obtained.

In order to check the linearity of response the light intensity was altered by varying the distance of the standard lamp from the photo-electric cell, with and without the zinc screen in place. The results are given in Fig. 2, and it is evident that there is no deviation from linearity up to 3,525 foot-candles, that

is an intensity in excess of the daily mean (dawn to dusk) at midsummer in the south of England.

Somers and Hamner (1951) have reported that there was an occasional failure in their counters, but no such defect was found with the type used in this instrument. In fact, apart from failures due to power cuts of the electricity supply, no difficulties in operation were encountered during either summer.

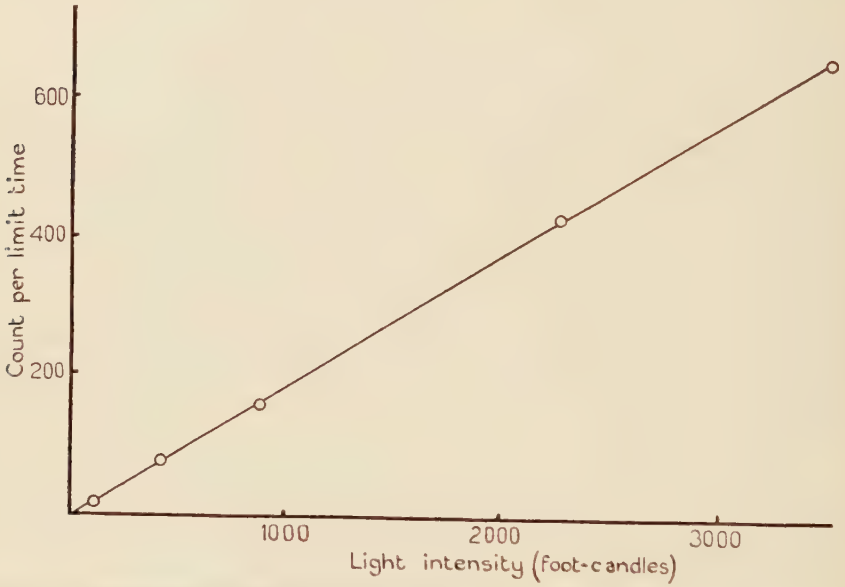

FIG. 2. The relationship between light intensity and the number of discharges registered per unit time by the recorder.

Lastly, it may be of interest to note that the cost of the components for the light recorder was about £25, of which a little more than half was for the photo-electric cell. Moreover, only normal workshop facilities are demanded for the construction of the boxes, chassis, &c. Although the design was related to a mains source of electricity supply, modifications could be made for its operation by batteries.

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Microsporogenesis in some Triploid Dactylorchid Hybrids

BY

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(*University College, London*)

With Plates XXXI and XXXII and two Figures in the Text

Abstract

During meiosis in naturally occurring triploid hybrids between the diploid *Orchis fuchsii* Druce ($2n = 40$) and the two tetraploids, *O. purpurella* Steph. and *O. praetermissa* Druce ($2n = 80$), there is a regular formation of 20 bivalents and 20 univalents. Since the two tetraploid species themselves show typical 'diploid' behaviour in synapsis and fertility, they are considered to be allopolyploids, and the hybrid pairing to be allosyndetic. The implication is therefore that both tetraploids are amphidiploids of which *O. fuchsii* has been one progenitor. It is suggested that varieties of the polytypic diploid *O. latifolia* L. sec. Pugsl. may have been the other progenitors. A feature of interest in the microsporogenesis of both parents and hybrids is the close synchronization of nuclear events in the pollen massulae, which behave as physiological units throughout meiosis and pollen-mitosis. In the triploids, although numerous dysploid nuclei are produced, none dies prematurely, probably because of mutual compensation within what is, in effect, a common cytoplasmic matrix.

Introduction

KLINGE'S two subdivisions of the genus *Orchis* L., *Euorchis* and *Dactylorchis*, are characterized by differences in karyotype as well as by various well-marked morphological features. From the investigations of Hagerup (1938*b*), Heusser (1938), and Vermeulen (1938, 1947) it appears that in the subgenus *Euorchis*, where polyploidy is unknown, the haploid numbers $n = 16$, 18, and 21 are found, and there is considerable diversity in chromosome size. In *Dactylorchis* (with the exception of *Orchis sambucina* L. which, with a haploid number of 21, occupies a special cytological position in the subgenus as well as being morphologically somewhat apart) the species investigated possess a base number $x = 20$, and the chromosomes are small and uniform. The diploid number, $2n = 40$, is found in two large form-complexes which appear to lie at the base of the system of variation in this subgenus. One comprises the group of diploid spotted orchids allied to *O. fuchsii* Druce, including the Madeiran endemic, *O. foliosa* Soland. The other embraces the numerous varieties and geographical races of *O. latifolia* L. sec. Pugsl. (*O. strictifolia* Opiz., *O. incarnata* auct. mult.) and closely associated species such as *O. cruenta* Müll. The remaining dactylorchids so far investigated are uniformly tetraploid ($2n = 80$), apart from the highly polymorphic

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O. traunsteineri Saut., in which plants with $2n = 40$, 80, and 122 have been recorded (Vermeulen, 1938, 1947).

It appears probable that the tetraploid dactylorchids, which form as complex a taxonomic group as is to be found amongst amphimictic flowering-plants in Europe, have arisen from *fuchsii*- or *latifolia*-like ancestors by processes of auto- and allopolyploidy, an hypothesis supported by their morphological affinities. Observations on the course of meiotic chromosome pairing in hybrids between the tetraploid species and their presumed diploid progenitors might be expected to throw light upon this matter. Cultivation difficulties have so far prevented controlled crossing experiments, but naturally occurring hybrids have proved a useful source of evidence. During the course of an extensive survey of the variation in the subgenus, cytological studies have been conducted on triploid ($2n = 60$) plants of the following presumed parentages from the localities indicated:

O. fuchsii Druce ($2n = 40$) × *O. purpurella* Steph. ($2n = 80$) Curragh Cloe, Co. Wexford; Westport, Co. Mayo.

O. fuchsii ($2n = 40$) × *O. praetermissa* Druce ($2n = 80$) Otterbourne, Hants; Gravesend, Kent.

The two tetraploid parental species concerned, *O. purpurella* and *O. praetermissa*, are practically vicarious in the British Isles (Heslop-Harrison, 1952*b*), and the hybrids were taken from mixed colonies of the presumed parents in localities where other genetical contamination would be highly improbable. The individual plants studied cytologically were selected for their morphological intermediacy, and their triploid status confirmed by examination of root-tip mitoses (Pl. II, A). It is presumed that all are of F_1 origin.

Although the taxonomic problems of some of the British tetraploid marsh orchids will shortly be dealt with in greater detail elsewhere, a note on the status of the var. *junialis* Vermln. of *O. praetermissa* Druce seems desirable here. This form has long been accepted by British systematists as the Linnaean '*O. latifolia*', and was described as the species '*O. pardalina*' by Pugsley at the time of his transference of the name *O. latifolia* to the plant previously known as *O. incarnata* (Pugsley, 1934). The view has occasionally been expressed that the form in question cannot be distinguished from the hybrid *O. fuchsii* × *O. praetermissa* (Druce, 1923; Clapham, Tutin, and Warburg, 1952). However, plants conforming with Pugsley's description of '*O. pardalina*' (and with the earlier one of *O. praetermissa* var. *junialis*, as *O. latifolia* var. *junialis*, Vermeulen, 1933) commonly occur in populations of *O. praetermissa* in districts where *O. fuchsii* is sparse or absent (Summerhayes, 1951). Plants of this type have been investigated cytologically from Sandwich, Kent; Sutton Broad, Norfolk; Greywell Fen, Hampshire; and Otterbourne, Hampshire, and all have been found to be tetraploid, $2n = 80$. In those in which meiotic studies have been possible, synaptic behaviour has proved to be perfectly regular; in fact this form, whatever taxonomic rank

may be ascribed to it, must be considered as quite stable cytologically and certainly not of recent hybrid origin. From the triploid (presumed F_1) hybrid *O. fuchsii* × *O. praetermissa*, which has also been collected from three of the above localities, the var. *junialis* can be distinguished morphologically by the less deeply divided labellum, thicker spur, deeper flower colour and patterning, larger stem cavity, smaller number of leaves, and the more acute lowest leaf.

Cytological Methods

Meiosis takes place in the anther when the bud is rather less than 3 mm. in length. Due to the slow acropetal development of the inflorescence, it is usually found that the apical buds are in this state while the lowermost flowers are opening. Plants can be determined taxonomically from these in ample time to permit collection of reducing anthers from the same inflorescence.

All material was fixed in Langlet's modification of Navashin's fluid. Pollinia were sectioned at 10–12 μ, and sections were stained in crystal violet by the method of Newton.

Meiosis in the Parental Species

Meiosis is regular in all of the parental species, and a careful search has failed to reveal multivalent configurations in either of the tetraploids. The meiotic behaviour of the latter is essentially of the 'diploid' type, which would seem to indicate that both are allopolyploids.

An interesting feature of meiosis arises from the fact that in *Orchis*, as throughout the tribe *Ophrydeae*, the microspores are not produced independently but cohere in massulae, aggregates of which form the characteristic pollinia. Certain results of this intimate association have been discussed by Barber (1942), who noted in '*O. maculata*' (a diploid form, probably *O. fuchsii*) strict synchronism in the events of pollen-mitosis between all of the nuclei of any particular massula. This he attributed to the fact that, due to the unthickened nature of the walls between the microspores, the nuclei, in effect, share a common cytoplasmic environment within the massulae. It appears that fairly strict intra-massular nuclear synchronism is quite characteristic also of the earlier meiotic events not discussed by Barber.

At the onset of the meiotic prophase, the 300- or 400- spore mother nuclei within the massula lie in a cytoplasmic matrix of a dense yellowish appearance, separated by walls of extreme delicacy. Already at this stage the massulae themselves lie separate in the anther, each surrounded by a film of tapetal fluid. This apparently imposes a certain amount of physiological isolation, for whereas within each massula there is close synchronization of development, neighbouring ones are commonly found to be at different stages (*O. fuchsii*, Pl. XXXI, A). Observations of preparations in anaphase stages suggest that there may often be a slight action gradient across the massula, since in dividing nuclei towards the outside (i.e. nearest the anther wall) groups of daughter chromosomes often show greater separation than in the innermost

ones. In the pollinium as a whole there is usually no very clear developmental gradient, although it occasionally appears to be the case that the last massulae to complete any stage of development are the smaller ones near the caudicle at what is morphologically the lower end of the anther.

Detailed studies of meiotic prophase stages are precluded by the large number and small size of the chromosomes, but at diakinesis it appears that terminalization of chiasmata is complete, and rod- and ring-bivalents are to be seen within the nuclear membrane. Diakinetic, metaphase, and late anaphase stages in *O. fuchsii* are illustrated in Pl. XXXI, A. As stated above, multivalent configurations are produced in none of the tetraploids, all of which regularly show 40 bivalents at metaphase I (*O. praetermissa*, Pl. XXXI, B). The remarkable extent of intra-massular synchronism is revealed in Pl. XXXI, C, which illustrates the completely regular anaphase I of *O. purpurella*.

The second meiotic division follows a normal course, again with a high degree of synchronization between all of the nuclei within the massula. Cytokinesis follows, but the walls formed between the microspores do not impair their developmental synchronism, since all nuclei within a particular massula pass simultaneously into the prophase of pollen-mitosis. The early stages of this division are normal in all respects, but, as Hagerup (1938*a*) has described for '*O. maculata*' (a diploid form near *O. fuchsii*), the later stages are distinctly asymmetrical. This asymmetry seems to be an extreme example of that often observed in pollen-mitosis in flowering plants in which the pollen is formed in independent tetrads (cf. Geitler, 1935; Darlington, 1936). The dividing nuclei in the outer microspores within a massula are regularly orientated so that the spindle axis is directed towards the centre, and during anaphase the outer group of daughter chromosomes is apparently arrested near the outer wall of the microspore while the inner group is carried inwards as the equatorial zone of the spindle extends. The daughter nuclei at telophase differ in appearance: the inner condenses immediately, while the outer remains for a while as a flat plate in which, in surface view, the individual chromosomes can be distinguished as in a metaphase (Pl. XXXI, D). Probably because of differences in fixation technique, it has not proved possible to confirm the corollary reported by Hagerup concerning the asymmetrical disappearance of the spindle, which is said to collapse towards the outer (generative) nucleus and away from the inner (tube) nucleus. The distributions of nuclear size in the mature pollinia in two of the taxa examined, *O fuchsii* and *O. purpurella*, are shown in Text-fig. 2*a* and *b*, and the mean diameters and volume ratios in Table I.

TABLE I. *Sizes of Pollen Nuclei* (mean diameters in μ)

	O. fuchsii	*O. purpurella*	Volume ratio *fuchsii* : *purpurella*
Generative ($N = 50$)	6·0±0·10	7·2±0·08	1·00:1·76
Tube ($N = 50$)	9·4±0·15	10·5±0·17	1·00:1·46

Meiosis in the Hybrids

No essential differences were observed in the meiotic behaviour of the two triploid hybrids, and the following account may be taken as applying to both.

Prophase. The synchroneity of development of the microspore mother nuclei, so characteristic of the parents, is equally apparent in the triploid hybrids. Bivalents and obvious univalents can be seen at diakinesis, but in no case has an exact count been possible.

Metaphase I. Good evidence of the nature of the synaptic reaction can be obtained from this stage, particularly since the developmental synchronism of the nuclei allows large numbers to be examined in almost exactly the same stage in favourably fixed massulae. Multivalent configurations have not been observed in any of the plants examined. It appears that the bivalents co-orientate first on the spindle equator, while the univalents remain randomly dispersed. Later the univalents self-orientate, so that eventually the appearance of the metaphase plates is as regular as in non-hybrid individuals. In a massula from a plant of *O. fuchsii* × *O. purpurella* from Co. Mayo 50 nuclei observed in polar view at this stage showed $20_{II}+20_{I}$, and the same distribution was noted in 46 metaphase-I plates counted in a plant of *O. fuchsii* × *O. praetermissa* from Gravesend, Kent (Text-fig. 1*a*), indicating that this pairing behaviour must be considered usual.

Anaphase I. The bivalents disjoin first, leaving the univalents distributed across the spindle equator; close synchronism is maintained between the nuclei within the massula (Pl. XXXII, B; Text-fig. 1, *b*). After the completion of the anaphasic movement of the bivalents, the univalents divide, and their daughter chromosomes move polewards (Text-fig. 1, *c*).

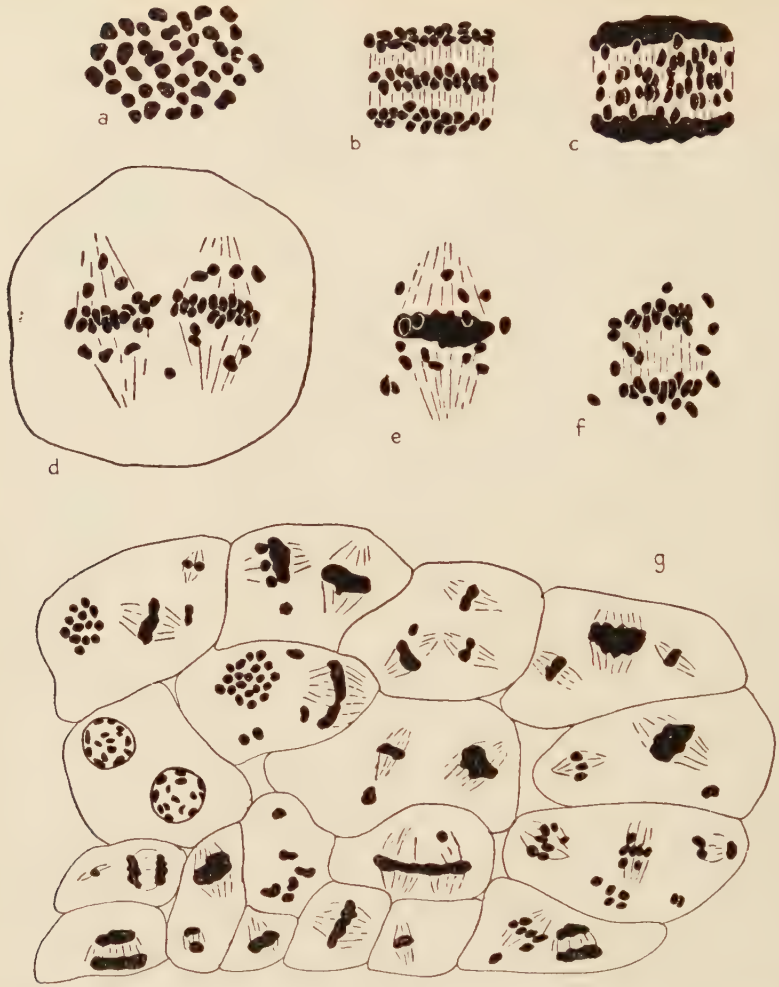
Telophase I. All of the daughter chromosomes resulting from the division of the univalents are incorporated in the reorganizing telophasic nuclei.

Metaphase II. Twenty chromosomes, presumably those resulting from the division of the univalents, show no sign of metaphasic orientation, and lie freely in the cytoplasm. The remainder (derived from the bivalents of meiosis I) take up a position on the spindle equator (Text-fig. 1, *d* and *e*).

Anaphase II. The chromatids of the paired chromosomes of meiosis I separate in the normal manner and proceed to the poles (Text-fig. 1, *f*). The univalents do not divide again.

Telophase II. Favourably placed univalents are incorporated in the reorganizing telophasic nuclei; the remainder form separate micronuclei singly or in small groups.

Pollen-mitosis. Up to this point the course of events is not markedly different from that found in other triploid hybrids in which multivalent synaptic configurations are absent. Commonly after meiosis of this nature the microspores in which the chromosome complement departs significantly from the balanced haploid or diploid condition abort, rarely proceeding as far even as pollen-mitosis. In these dactylorchid hybrids, however, the physiological inter-dependence of the cells of the massulae evidently compensates



TEXT-FIG. 1, *a–g*. Meiosis and pollen-mitosis in triploid hybrids: *a* and *c*, *O. fuchsii* × *O. praetermissa*, remainder, *O. fuchsii* × *O. purpurella*. *a*, Metaphase I showing $20_{II}+20_{I}$. *b*, Anaphase I; 19 of the 20 expected univalents distinguishable. *c*, Later anaphase I; daughter chromatids of the univalents moving polewards. *d*, Metaphase II; bivalents of meiosis I oriented across the spindle-equator, univalents irregularly distributed in the cytoplasm. *e*, Similar stage; 17 of the 20 expected univalents discernible. *f*, Anaphase II. *g*, Pollen-mitosis in a segment of one massula. Stages visible from late prophase to telophase, with dividing nuclei containing from 1 to 20 chromosomes.

(*a* × *c*. 2,500; *b–f* × *c*. 2,000; *g* × *c*. 1,750)

for the genetical unbalance of the various nuclei which results from their dysploidy. At the stage of pollen-mitosis most nuclei, including those which incorporate only a single chromosome, attempt division. The close synchronization of the earlier phases is lost to some extent, and within a single massula stages of division ranging from prophase to telophase may be seen

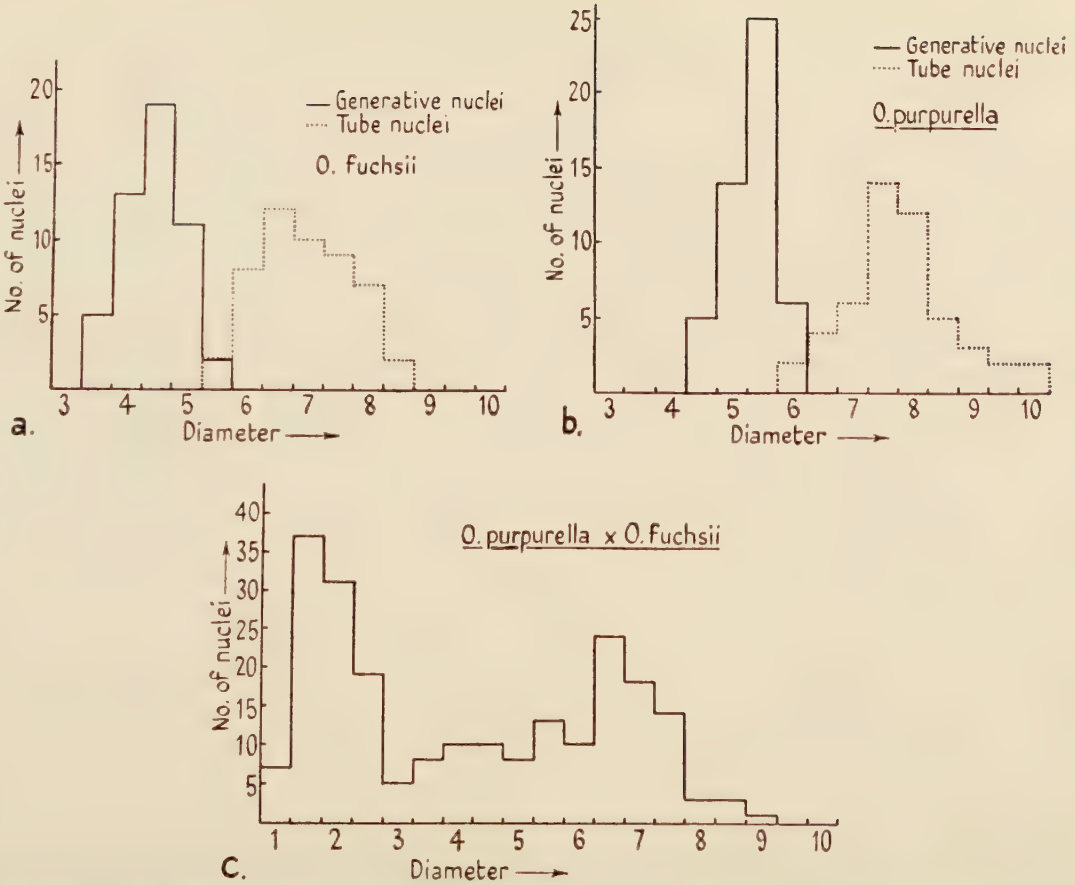
(Pl. II, c; Text-fig. 1, *g*). During prophase a condition is reached which simulates meiotic diakinesis, with extremely contracted chromosomes lying well separated near the nuclear membrane (centre left, Text-fig. 1, *g*). The rather regular centrifugal orientation of the spindle axes noticeable in the pollen-mitoses of the parents is absent in the majority of the hybrid nuclei, although a few (presumably in those microspores in which the chromosome complement is balanced) show the characteristic asymmetrical telophase. The larger metaphase plates often appear curved, as though as a result of space limitation within a malformed microspore, and possess distorted or multipolar spindles (examples in both Pl. XXXII, c, and Text-fig. 1, *g*).

At the completion of pollen-mitosis the cells of the massulae contain from 2 to 7 nuclei (Pl. XXXII, d). Since a considerable proportion of the cells contains odd numbers, more than might reasonably be accounted for by counting errors, it appears that some pollen-mitoses must fail, or end in the production of restitution nuclei. The size distributions of the nuclei in 50 cells of a ripe massula of *O. fuchsii* × *O. purpurella* is shown in Text-fig. 2, *c*. The distribution is markedly bimodal, and it would appear that this is readily enough explained from the previous course of meiosis and pollen-mitosis. Presumably the first mode represents the micronuclei, many probably containing no more than a single chromosome, derived from the univalents of meiosis I, while the second represents those nuclei which incorporate in the neighbourhood of 20 chromosomes, those arising from the regular second division of the synapsed chromosomes of meiosis I. All nuclei in the hybrid pollinium appear to remain in a diffuse state corresponding to that of the tube nuclei in the parents.

At the time of anthesis the pollinia of the hybrids differ little from the normal in macroscopic appearance, and they can be detached from the thecae with little difficulty. In an experimental pollination of a plant of *O. fuchsii* with pollinia from the hybrid *O. fuchsii* × *O. purpurella*, a reaction was produced similar to that resulting from pollination from a normal plant. The flower began shortly to wither and the ovary to swell with consequent rotation of the previously resupinate flower as the 180° twist became eliminated. If this reaction is due to a hormonal stimulus from the pollinium, it implies that the hybrid pollinium, despite its great nuclear irregularity, retains some of the physiological properties of the normal one.

The Origin of the Tetraploid Marsh Orchids

The synaptic behaviour which produces $20_{II}+20_{I}$ in these triploid hybrids can, of course, be interpreted either as auto- or allosyndesis. In the former case the chromosomes originating from each of the tetraploid parents would be pairing *inter se* in their respective hybrids, leaving the haploid set derived from *O. fuchsii* as univalents; in the latter case the *fuchsii* set would be pairing with a homologous set present in each tetraploid, leaving the remaining chromosomes of the tetraploids as univalents. The numerical relations of the chromosome complements involved, and the lack of appreciable differences



TEXT-FIG. 2, *a–c*. Sizes of nuclei in pollen. *a*, *O. fuchsii*; *b*, *O. purpurella*; *c*, *O. fuchsii* × *purpurella*. 50 cells examined in each case; 1 scale-unit = 1·35 μ.

in size or morphology which might have provided some guidance, eliminate the possibility of any decision on the matter from cytological observations of the hybrids alone. However, as stressed above, the tetraploids concerned show 'diploid' type of behaviour in their meiosis, with complete absence of multivalents. This would appear to indicate a relatively low degree of homology between their component chromosome sets, although it does not entirely exclude the possibility of a synaptic reaction between them in the presence of a totally foreign set in a hybrid.

However, if allosyndesis does occur in these triploid hybrids, it implies that both of the tetraploid marsh orchids concerned are amphidiploids of which *O. fuchsii* has been one parent, and if morphological attributes are taken into account there is much to support this hypothesis. The fairly obvious affinities of *O. purpurella* and *O. praetermissa* with *O. maculata* agg. (of which *O. fuchsii* is a segregate) have prompted various authors to suggest that they are hybrid derivatives from the latter (Pettersson, 1947; Summerhayes, 1951), and a possible allopolyploid derivation for these and other tetraploid marsh orchids has, in fact, already been suggested (Vermeulen, 1947; Heslop-Harrison, 1952 *a*).

The presumption that *O. purpurella* and *O. praetermissa* are amphidiploids both of which have had as one diploid progenitor *O. fuchsii* invites speculation as to what, in each case, has been the other. There is only one other series of diploid forms which would appear to have been at all qualified for the role, namely, that formed by the numerous varieties of *O. latifolia* (*O. incarnata* auct. mult.). The tetraploid marsh orchids themselves, although ranked taxonomically as 'species', are in actuality no better differentiated from each other morphologically than are 'varieties' of *O. latifolia* such as var. *gemmana* Pugsl., var. *pulchella* (Druce) Pugsl., and var. *coccinea* Pugsl. It would not appear to be beyond possibility that these or similar forms have been the other progenitors of the tetraploid marsh orchids, and, indeed, that what small amount of genetical differentiation exists amongst the latter has resulted from genetical differences between the *latifolia* parents which have participated in each hybridization from which an amphidiploid has arisen, rather than from the *fuchsii* parents. It is to be expected that critical evidence might be forthcoming from meiotic studies of hybrids between the tetraploid marsh orchids and the *latifolia* forms, or between the latter and *O. fuchsii*. These hybrids are, unfortunately, far rarer in nature than those discussed in this paper, and as yet none has been obtained in a suitable state for cytological study.

Summary

The course of microsporogenesis has been followed in *Orchis fuchsii* Druce ($2n = 40$), *O. purpurella* Steph. ($2n = 80$), and *O. praetermissa* ($2n = 80$), and in natural triploid ($2n = 60$) hybrids between the first species and the latter two.

Meiosis is completely regular in each of the three taxonomic species, and

also in the var. *junialis* of *O. praetermissa* ('*O. pardalina*' Pugsl.), a form sometimes stated to be a hybrid. There is close synchronism in the events of microsporogenesis within a single massula, but not between the different massulae of a pollinium.

The meiotic behaviour of presumed F_1 hybrids of the parentages *O. fuchsii* × *O. purpurella* and *O. fuchsii* × *O. praetermissa* is essentially similar. Multivalents are not formed, and there is a regular appearance of 20 bivalents and 20 univalents at metaphase. The univalents divide in meiosis I, but not in meiosis II, at the close of which they are incorporated at random in the telophasic nuclei or form independent micronuclei. All nuclei survive until pollen-mitosis irrespective of their chromosome number, a result attributed to their physiological co-operation within the massula. The pollinium of at least one of the hybrids is capable of promoting the growth of an ovary when applied to the stigma of another plant.

If the pairing behaviour which produces $20_{II}+20_{I}$ in the triploid hybrids is interpreted as allosyndesis, the implication is that *O. purpurella* and *O. praetermissa* are both amphidiploids of which *O. fuchsii* has been one progenitor. It is suggested that the other progenitors might have been varieties of the polytypic *O. latifolia* L. sec. Pugsl. (*O. incarnata* auct. mult.).

Acknowledgements

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EXPLANATION OF PLATES

Illustrating J. Heslop-Harrison's article on 'Microsporogenesis in Some Triploid Dactylorchid Hybrids'.

PLATE XXXI

Microsporogenesis in dactylorchid species.

A. Pollinium of *O. fuchsii*. Late diakinesis in the massula at bottom left, metaphase I in that at top right, and anaphase I in that in centre. (×*c.* 600.)

B. Metaphase I in *O. praetermissa*, showing 40 bivalents. (×*c.* 1,400.)

C. Anaphase I in *O. purpurella*. (×*c.* 1,400.)

D. Pollen-mitosis in *O. purpurella*; metaphase. (×*c.* 1,500.)

E. Pollen-mitosis in *O. purpurella*; telophase, showing the asymmetrical nature of the division. (×*c.* 1,500.)

PLATE XXXII

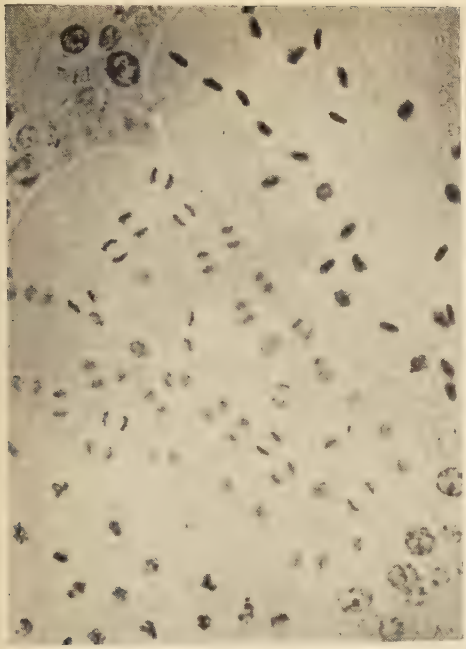
Cytology of the triploid hybrid, *O. fuchsii* × *O. purpurella*.

A. Root-tip mitosis, $2n = 60$. (×*c.* 2,000.)

B. Anaphase I, showing separating bivalents and univalents distributed across the spindle-equator. (×*c.* 1,800.)

C. Pollen mitosis (cf. Text-fig. 1, *g*). (×*c.* 1,200.)

D. Mature pollinium showing micronuclei and macronuclei. (×*c.* 1,500.)



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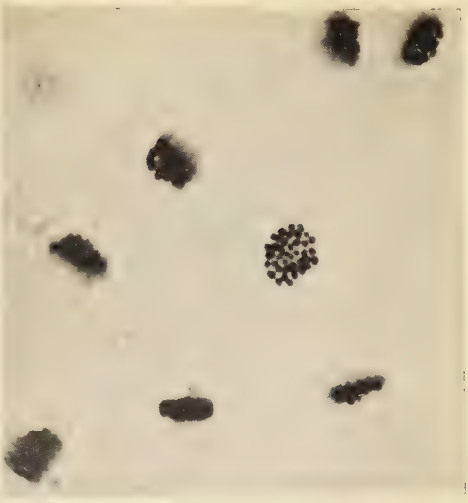

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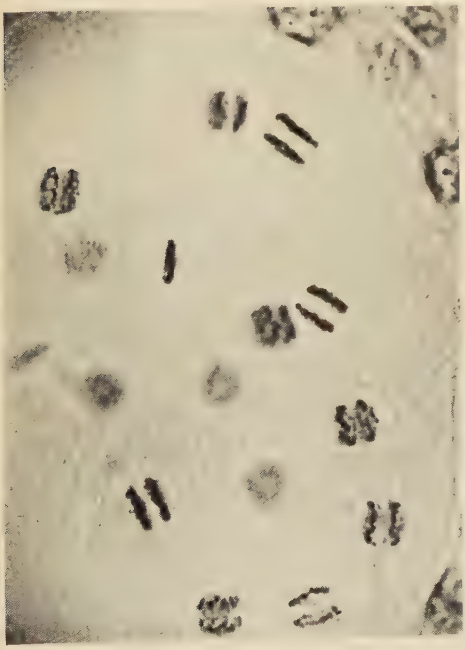

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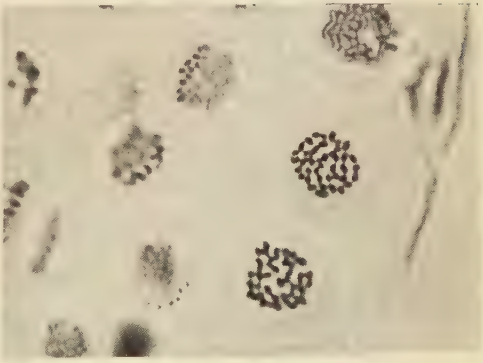

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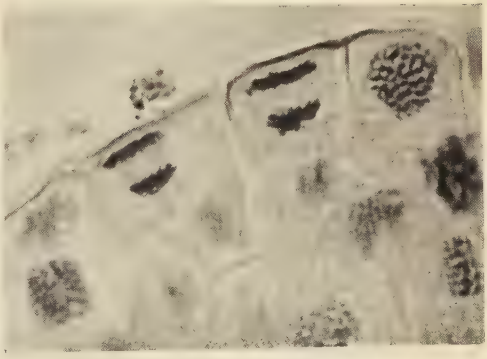

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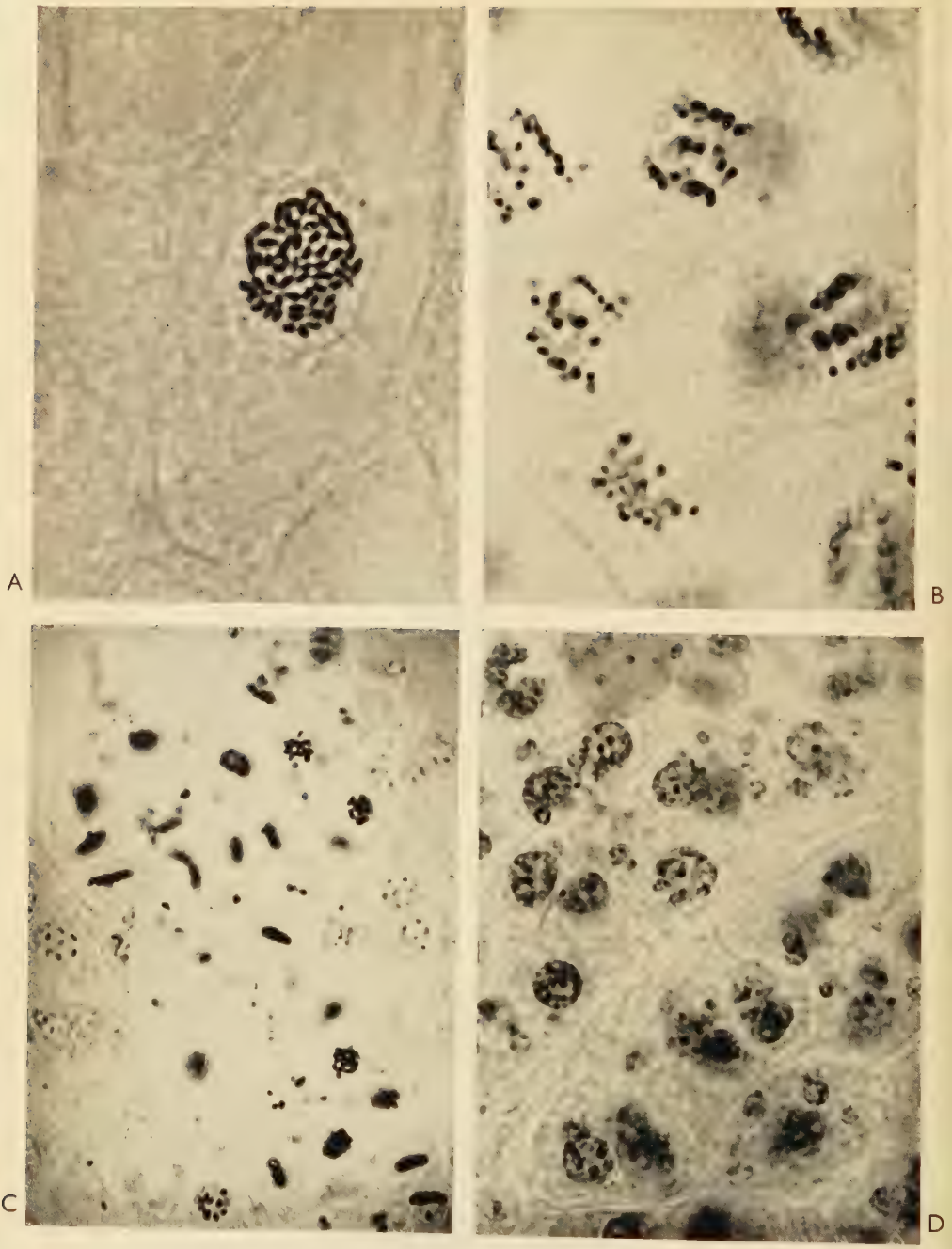
HESLOP-HARRISON



HESLOP-HARRISON

Studies on the Systemic Fungus, *Puccinia suaveolens*

BY

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With Plate XXXIII and six Figures in the Text

Abstract

The autoecious rust *Puccinia suaveolens* attacks the creeping thistle *Cirsium arvense*. Infection with uredospores first produces isolated uredo-pustules, usually with teleutospores. The mycelium later grows down into the roots and becomes systemic. In some of the root-buds the mycelium appears unchanged and bears uredosori; in others it undergoes a somatic segregation of mating type and bears spermogonia only. Teleutospore germination is so erratic that basidiospore infection is responsible for only a small amount of the spermogonial infection observed.

The spermogonial mycelium in the systemically infected shoots is heterothallic and there are two mating types. All the spermogonia on one shoot bear spermatia of only one mating type. Therefore segregation of mating type must take place at the base of the shoot or even in the roots. The multinucleate condition of the mycelium in both uredosoral and spermogonial shoots makes it impossible to observe segregation directly.

Part I. *Establishment of the Systemic Mycelium and Investigations into its Heterothallism*

Introduction

THIS paper deals with the results of an investigation into the biology of *Puccinia suaveolens* (Pers.) Rostr. The history and biology of this fungus have already been reviewed by Buller (1950). Buller's account is not always complete and is at times based on slender evidence. The observations and experimental results reported below should widen knowledge of the life-history of this fungus.

The life-history of the fungus. Puccinia suaveolens is an autoecious brachy rust which shows two distinct types of infection:

1. Localized infection in which isolated pustules of uredospores and teleutospores are found on the leaves.
2. Systemic infection in which whole shoots bear either spermogonia or uredosori, sometimes with teleutospores.

It is the relationship between the two types of infection which forms the central problem.

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In 1941 Buller and Brown reported that the systemic mycelium of *P. suaveolens* could be established by inoculating the first foliage leaves or cotyledons of seedling thistles with uredospores. As a result secondary uredosori developed on the inoculated leaves. After some months, buds on the roots of the inoculated plants grew into infected shoots the leaves of which bore spermogonia usually followed by primary uredosori. One systemically infected shoot bore spermogonia only and thus enabled these workers to demonstrate heterothallism. In their words (1941), 'when nectar from other infected plants was mixed with the nectar of the shoot that bore pycnia only, uredospores formed at the point of mixing. This indicates that *P. suaveolens* is heterothallic.'

Following on Buller and Brown's discovery (1941), Professor Brooks at Cambridge artificially infected naturally occurring thistle seedlings with uredospores in 1941 and again in 1944, and found that systemically infected shoots developed. In the spring of 1947 the experiments were carried out on a larger scale with thistle seedlings raised from seed. Of the 30 seedlings inoculated, 14 subsequently became systemically infected.

Establishment of the Systemic Mycelium

Inoculations were begun by the writer in 1948. Uredospore and teleutospore material for inoculations was collected from a number of localities near Cambridge. Although most of the inoculations were made with uredospores, for the sake of simplicity those in which teleutospores were used will be described first.

Infection by basidiospores. The teleutospores of *P. suaveolens* are notoriously difficult to germinate. Buller and Brown (Buller, 1950) found it impossible at first; later, spores overwintered in a refrigerator were seen to germinate after 24 hours at room temperature to give a four-celled basidium bearing four basidiospores. With these basidiospores they produced localized spermogonia on thistle seedlings in 10 to 12 days. Whether the infections would have eventually become systemic is not known for the plants were lost.

Early attempts by the writer to germinate teleutospores failed. Subsequently teleutospores which had been stored during the winter at 5° C. and then kept moist at room temperatures for about 6 weeks were seen to germinate, but very erratically. Of approximately 16,000 spores examined, 159 germinated, producing basidia but no basidiospores.

Since no basidiospores could be obtained for direct infection, the teleutospores themselves were used as inoculum. Leaves bearing teleutosori were gathered in the autumn and kept in a refrigerator at 5° C. In the spring, when tests were made prior to inoculation, it was found that many of the accompanying uredospores were still capable of germination. When the teleutospore material was removed from the refrigerator and kept at room temperatures for about 6 weeks, the uredospores were rendered completely inviable whereas the teleutospores still retained their viability, as indicated by their

appearance under the microscope and the fact that they could still be plasmolysed.

Inoculations with these 'after-ripened' teleutospores were carried out as follows. Thistle seed was sown in pots of sterlized soil, and at the same time chopped up thistle leaves bearing teleutospores, treated as above, were strewn thickly over the surface of the soil. The pots were covered with sheets of damp filter-paper and kept in a moist atmosphere under bell-jars until the seedlings were well developed. It was hoped that this prolonged wetting would cause some of the teleutospores to germinate. After about 6 weeks the pots of seedlings were removed to greenhouse benches and protected from stray infection by cellophane cages.

Two experiments were carried out. In the first, 80 plants were present, of which 6 became systemically infected. Two of these showed spermogonial infection of the main shoot, two spermogonial infection of side shoots, and two uredosoral infection of side shoots. In the second there were 100 plants, of which 1 had uredosoral infection of a side shoot.

As uredospore infection can be discounted in these experiments, it appears that basidiospore infection occurs and produces systemic infection of both main and side shoots. The uredosoral infections found were presumably the result of infection of a shoot by basidiospores of both mating types. It is difficult, however, to estimate how great a part this type of infection plays in the life-history of the fungus. Infection by basidiospores would be expected to give rise first to a localized spermogonial mycelium which would later become systemic. As pointed out by Buller (1950), such young, isolated spermogonial pustules would be easily overlooked. Buller himself discovered only one, a few millimetres in diameter. In the present work, also, only a single isolated pustule was found on the many plants examined: microscopical investigation showed that the mycelium was strictly localized, thus suggesting that it was the product of a single basidiospore infection. The erratic nature of teleutospore germination, however, makes it unlikely that much of the systemic spermogonial mycelium arises from basidiospores. As first shown by Buller and Brown (1941) and later confirmed by Professor Brooks, spermogonial infection does arise very abundantly from the systemic mycelium produced by infection with uredospores.

Infection of uredospores. Primary uredospores, when inoculated on to healthy plants, always produce secondary uredosori localized at the points of infection. This secondary uredospore generation can be repeated indefinitely. Inoculations were carried out on seedlings, adult plants, and root-buds.

Seedlings were inoculated with uredospores on the lower sides of the cotyledons. Infection occurred very readily, secondary uredosori appearing on all inoculated leaves about 2 weeks later. Systemic infection followed in about 45 per cent. of the plants inoculated, the first infected shoots appearing later in the same year in plants inoculated early in the season, or in the following spring in those inoculated later (Table I).

Adult plants were successfully infected by spraying them three or four times

with a dense suspension of uredospores. Systemic infection followed in 29 of the 185 plants inoculated.

TABLE I. *Inoculations of Seedlings with Uredospores*

No. of seedlings inoculated between Mar. and June 1948	147
,, ,, plants showing systemic infection in same season	50
Total No. of seedlings inoculated in 1948	194
,, ,, ,, plants showing systemic infection by May 1949	88

Thirty root-buds were transplanted from plants which had been carefully protected from any sources of infection and inoculated with uredospores. Two of them developed systemic infection.

Nature of the systemic infection. The following account is based on observations made on plants inoculated as seedlings with uredospores.

After the development of the secondary uredosori the mycelium generally travels out of the cotyledons into the stem and down into the roots. Only one of the several thousand seedlings inoculated developed systemic infection of the main shoot. Buller (1950), on the other hand, found systemic infection of the main shoot to be much more common. The axillary shoots arising at the base of the main stem were often the first to show systemic infection. As the plants became older, infected shoots developed from roots farther away from the main shoot. The continuity of this mycelium with that of the main stem was easily determined by sections of the intervening roots. Both kinds of systemic mycelium, that producing only spermogonia at first and that producing only uredosori, appear on most plants at some time in their life-history. In order to provide evidence of the relative frequencies of the two kinds, a set of 60 plants has been chosen. These had been inoculated with uredospores in the spring of 1948 and had shown systemic infection in the same season (Table II).

TABLE II. *The Nature and Extent of the Systemic Infection Produced after Inoculation with Uredospores*

No. of plants showing systemic infection	60
No. in which the first infected shoot to appear bore spermogonia	34
,, ,, ,, ,, ,, ,, uredosori	26
No. of plants which produced 1 or more spermogonial shoots during the experimental period	59
Total No. of systemically infected shoots produced by May 1949	191
Total No. bearing spermogonia only	106

HETEROTHALLISM OF *PUCCINIA SUAVEOLENS*

During 1948 and 1949 isolated spermogonial infections were established by taking cuttings of spermogonial shoots (from plants inoculated as seedlings with uredospores) as soon as they appeared above ground. Precautions were taken to avoid the transfer of spermatia during this operation. The cuttings were repotted in sterilized soil and covered with cellophane cages to prevent the access of insects. Such cuttings remained in the spermogonial condition

as long as they survived (in some cases up to 12 weeks) or until they were used for experiments.

Spermatization experiments with mixed spermatia. A suspension of spermatia was collected by wiping a moistened, sterile, camel-hair brush over the surface of a spermogonial shoot and dipping it in a tube containing a few drops of sterile water. Collections were made from seven shoots, using a fresh brush for each leaf. The suspension was then applied to marked leaves on five other shoots. These shoots were then incubated under separate bell-jars for 48 hours and then returned to the same cellophane cages.

Uredosori appeared 8–12 days later on six of the nine leaves so treated. None appeared on any of the leaves used as sources of spermatia. This experiment thus give strong support to the belief that *P. suaveolens* is heterothallic.

Some 5 days after uredospores first appeared on the treated leaves they began to develop on stems and leaves above. On the leaves sori appeared first along the midribs and then gradually spread out over the lamina. Spermogonia production did not cease immediately after spermatization, and on some shoots active spermogonia could still be seen on newly developed leaves as long as 30 days later. The spermogonia were usually in small patches, making it appear that spermatization of that part of the mycelium had not occurred. Eventually, however, the newly formed leaves produced nothing but uredospores. On the other hand, uredospore production did not proceed down the shoots for more than a millimetre or two below the lowermost inoculated leaf. One plant from this experiment later produced small side shoots from root-buds. These bore spermogonia only and had evidently been invaded by the unspermatized mycelium from the base of the main shoot. These side shoots continued to produce only spermogonia until they were preserved some weeks later.

Since no uredosori were developed on the leaves used as sources of spermatia it follows that all the spermogonia on one leaf are of the same mating type. The next experiment was designed to show whether this was also true of all the spermogonia on one shoot. A number of shoots were selected; spermatia were transferred from leaf to leaf within the same shoot, a separate brush being used for each shoot. In no case did uredosori appear on any treated leaf.

Two further experiments were carried out with a mixed inoculum from 10 shoots. The first, second, and sometimes the third leaf from the top of 15 shoots were spermatized. Uredospores appeared on one or more of the leaves of all the shoots inoculated; none appeared on any of the shoots used as sources of spermatia. In all these experiments it was noted that uredospores appeared in the greatest profusion on the younger leaves treated, suggesting that the spermogonia are receptive for only a limited time (Text-fig. 1).

Spermatization experiments with spermatia of a single mating type. If the heterothallism of *P. suaveolens* is of the one-locus, two-allelomorph type usual in the Uredinales (Whitehouse, 1949), then when spermatia of one mating

type are applied to a random sample of spermogonial shoots the ratio of positive to negative spermatizations should be approximately 1:1. In the first experiment a spermogonial shoot (No. 614) which had been grown from a cutting some time beforehand was used as the source of spermatia. A suspension of spermatia was collected from this shoot in the usual way and applied to the leaves of a number of spermogonial shoots, great care being taken to use a separate brush for each leaf. Ten days later uredospores appeared on inoculated leaves of 5 of the 12 shoots so treated. The ratio of 5 positive to 7 negative spermatizations agrees well with the expected 1:1 ratio.

Shoot 614 was too old for further experiments and was replaced by another one, No. 531. When the new shoot was crossed with the old tester, 614, the reaction was positive, indicating that the two shoots bore spermogonia of opposite mating type. As cuttings of new spermogonial shoots were established, they were tested against shoot 531 for mating type. The results of these tests were 15 positive to 22 negative spermatizations.

For purposes of comparison the mating type of the tester spermogonial shoot 614 is taken arbitrarily as negative. Thus the mycelium of a spermogonial shoot which produced uredosori when treated with spermatia from this tester shoot is positive. The second tester, 531, which gave a positive reaction to shoot 614, is therefore positive. All shoots which gave a positive reaction to shoot 531 are thus negative on the arbitrary scale and vice versa, assuming there were no instances of a failure to produce uredospores due to causes other than identity of mating type. The mating type ratio of all the shoots tested in 1949 is therefore 27+ to 22—. A x^2 test gives $x^2 = 0{\cdot}51$ and $p = 0{\cdot}5$ approx., indicating a good fit with a 1:1 ratio.

When a number of spermogonial shoots from any one parent plant were tested they were usually found to bear spermogonia of the same mating type. Since many more shoots were often tested from some plants than others, a better estimate of the mating-type ratio can be made by grouping the shoots according to the plant from which they were derived and assigning a mating type to the parent plant. If the above shoots are lumped in this way and the three plants which showed variable reactions are omitted, the mating-type ratio is 12+ to 13—.

Variable reactions may be due either to the presence of spermogonial shoots of both mating types on the one plant or to experimental error, e.g. not enough inoculum applied to or few active spermogonia on the inoculated leaves. Temperature possibly also influences spermatization: it is difficult to obtain any positive spermatization after mid April although spermogonial shoots are still being produced. The fact that experimentally negative results outnumber positive suggests that the discrepancies in the ratios might be attributed to experimental error.

In view of the fact that many of the infected plants were likely to bear shoots of only one mating type, whole plants were covered in the spring of 1950 and the spermogonial shoots tested as they appeared. Tester shoots were chosen from plants which at the time of the experiment bore only one

strong spermogonial shoot. Three such testers were used, two of which came from the same plant. Table III gives the reactions of the spermogonial shoots tested against these testers.

TABLE III. *Summary of Spermatization Experiments in 1950*

Testers	1976*a*		1976*b*		1532	
	+	−	+	−	+	−
Ratio of reactions of *shoots* to each tester .	27	24	7	6	17	34
Ratio of reactions of *plants* to each tester .	12	12	5	5	8	20

When the shoots are lumped together according to the parent plant and the plants which showed a variable reaction omitted (2 in tests against 1976*a* and 4 against 1532), the ratios are much closer to unity (Table III). The large number of negative reactions to 1532 is probably due to the fact that the tests were carried out late in the season.

PART II. *Cytological Studies of the Systemic Mycelium*

MATERIAL AND METHODS

Leaves to be embedded in paraffin wax were fixed in chrom-acetic urea (Allen, 1927) for 24 hours and embedded in ceresin paraffin (as supplied by British Drug Houses) to which had been added 4 per cent. of a crude rubber mixture prepared according to Johansen (1940). The most successful stain for embedded material was 1 per cent. aqueous safranin, which stained the fungal nuclei a brilliant red, and fast green as a counter stain. A distinction could be made between the nuclei of actively growing cells and senescent ones. In the former there was a brightly staining nucleolus surrounded by a clear area, while in the latter all the nucleus took up the stain.

A useful method was developed by which, by a process of hydrolysis, much of the leaf tissue could be removed, leaving the fungus more or less intact. Material fixed in formalin-alcohol (3 per cent. commercial formalin in 70 per cent. alcohol) gave the best results. Sections were cut about 30μ thick, hydrolysed in normal hydrochloric acid at 60° C. for 15 minutes, and stained in Giemsa solution (Gurr) for 2 minutes. Since Giemsa is particularly sensitive to pH the sections were washed in a phosphate buffer of pH approximately 7 (Bolles Lee, 1937), both before and after staining. The sections were then mounted under a cover-slip and tapped sharply with a needle to macerate them. The leaf cells broke apart and fell away, leaving the fungus more or less intact. The nuclei of the fungal hyphae stained reddish blue and stood out clearly against a bluish background, especially in the young hyphae (Pl. XXXIII, Fig. 2). Unfortunately, however, both the developing spermogonium and the uredosorus stained so deeply by this method that no internal structure could be made out. Material fixed in acetic alcohol gave the best squash preparations of this stage, but none of the many stains tried, e.g. acetic carmine, acetic orcein, acetic lacmoid, and Feulgen, together with various mordants, would stain the nuclei.

Whole plants were usually fixed in formalin alcohol. Cotton blue in

lactophenol was used for quick tests for the presence of the fungus in stems and roots. It is not a precise stain since it stains also the host cell contents, but with practice the fungal haustoria could be readily distinguished even in cross-section.

Cytological Investigations

Development of the secondary uredo mycelium after infection with uredospores. Uredospores germinate readily on cotyledons or other leaves. The germ-tube grows towards a stoma where it produces a club-shaped appressorium. The uredospore itself is binucleate and on germination the two nuclei migrate into

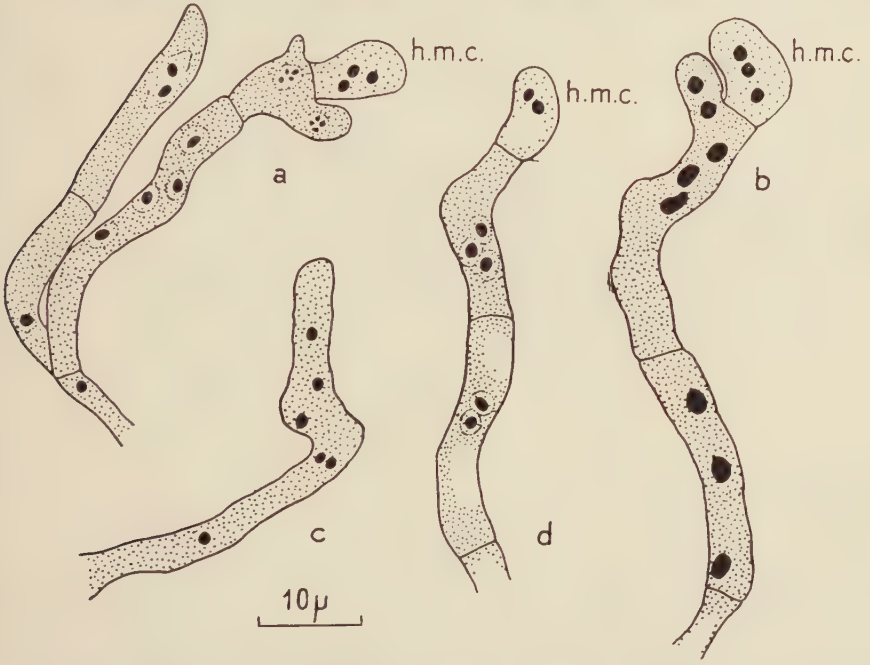
Text-fig. 1. Squash preparation of the fungus entering the host leaf. *a*, appressorium; *g.c.*, guard cells; *h.m.c.*, haustorial mother cell; *s.v.*, substomatal vesicle.

the germ-tube where they lie one behind the other a short distance from the tip. The nuclear constitution of the appressorium has not been ascertained. From the appressorium a tube is put forth which pushes its way between the guard cells of the stoma. Preparations from hydrolysed leaves show the tube to be a large, thin-walled structure, easily squashed by the pressure of the guard cells (Text-fig. 1). Inside the host the hypha swells out again to form the substomatal vesicle. The number of nuclei in the vesicle is 3. From it develops the primary infection hypha, again with 3 or sometimes 5 nuclei. As soon as this hypha comes near a mesophyll cell an haustorial mother-cell is cut off. The nuclei of the haustorial mother-cells vary from 2 to 5. When the terminal growth of the hypha is stopped by haustorial formation, one or sometimes two branches are produced by the subtending cell. The most striking feature of these cells and also of normal hyphal tips is the large number of nuclei present, ranging from 4 to 6 (Pl. XXXIII, Fig. 2; Text-fig. 2, *a*, *b*, and *c*). As far as can be ascertained, non-dividing cells usually contain either 2 or 3 nuclei per cell. One hypha may consist of all binucleate cells, all trinucleate or sometimes mixed bi- and trinucleate (Text-fig. 2, *d*). The large numbers of nuclei at the tips of the hyphae must therefore be the result of nuclear division without wall formation. The life of older, non-dividing cells is apparently so short that it is difficult to determine the relative frequencies of binucleate and trinucleate cells in the mycelium. The nuclear content of a number of hyphal tips have been scored with the following result (Table IV).

TABLE IV. *The Nuclear Content of Hyphal Tips*

Number of nuclei per cell	3	4	5	6
Number of cells	3	18	12	3

The four-nucleate stage may be produced by the division of both nuclei of a binucleate cell. The five-nucleate stage may then be an intermediate in the division of a trinucleate cell, that is, after the division of two nuclei



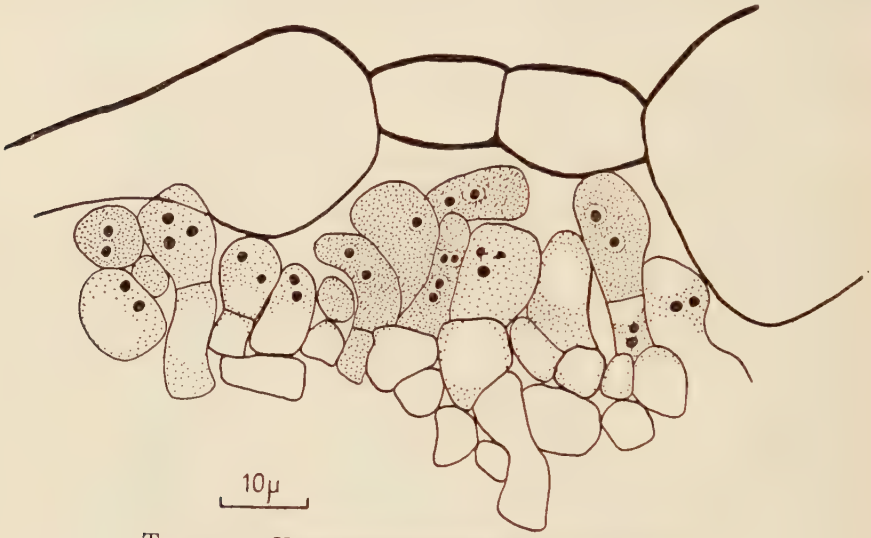
TEXT-FIG. 2. Hyphae of the 'Dikaryophase' with more than two nuclei. *h.m.c.*, haustorial mother cell; *a*, *b*, and *c* are from sections and *d* from a squash preparation.

but before the division of the third. It may be that the third does not divide so regularly, thus accounting for the low frequency of six nucleate cells and the occurrence of adjacent bi- and trinucleate cells in one hypha.

Formation of the secondary uredosorus. The secondary uredo mycelium is relatively slow growing: a colony 16 days old had a diameter of only 1·5 mm. The secondary uredosorus develops in the middle of the colony close to the point of origin of the infection. At an early stage a number of hyphae with dense contents grow across a substomatal cavity towards a stoma. The first hyphae to reach the cavity remain sterile and act as a cushion, forcing up the overlying epidermis. The fertile, spore-forming hyphae are a later invasion pushing aside the sterile cells. Although so much of the vegetative mycelium is trinucleate, the majority of the cells in the uredosorus primordium are binucleate (Text-fig. 3). Trinucleate cells do occur, but they usually have thin contents and passive nuclei and are evidently not fertile, possibly because of

their unbalanced nuclear content. Uredospores develop from the fertile hyphae in the usual way. Teleutospores usually appear later: their stages of development follow the normal sequence. When first formed they consist of two binucleate cells but the paired nuclei fuse before the spores are shed.

Passage of the mycelium into the roots. After passing out of the inoculated leaves the uredo mycelium first travels down the outer cortex of the hypocotyl. It grows very slowly, e.g. 5 mm. in 16 weeks in one plant, and is composed of stunted hyphae closely packed into the small intercellular spaces of the cortical parenchyma. Farther down it moves into the inner cortex and phloem and there begins to grow rather faster. In the phloem of the root the fungus forms a copious mycelium which can be easily detected in sections. It occurs less commonly in the xylem, probably because the densely packed, heavily lignified tissue offers too great resistance to intercellular growth. A few hyphae have been detected actually pushing between the vessel segments. The cells of the root mycelium are much shorter and thicker than in aerial parts and their nuclear content shows considerable variation both from cell to cell and from hypha to hypha (Text-fig. 4). The nuclei of 203 cells in the mycelium of a root were counted, care being taken to score only those cells whose end walls could be plainly seen and to neglect hyphal tips (Table V).



TEXT-FIG. 3. Vertical section through a young uredosorus.

TABLE V. *The Nuclear Content of Cells in a Root Mycelium*

Number of nuclei per cell	1	2	3	4	5	6	7
Number of cells	8	59	79	47	8	1	1

It was not often possible to score a series of cells in one hypha since growth of the mycelium in roots is so contorted. The few that could be scored were: 2–2–2–4; 3–2–3; 3–4–1–4–3–3–4; 2–2–2–; 2–4–3; 2–1–1–2; 4–3–4; 3–3–2;

6–4–3; 4–2–3; 3–2–3; 2–2–2; 2–7–2; 3–4–5; 5–4–4–4–2. This multinucleate condition was found in all plants examined and suggests that the dikaryotic phase of *P. suaveolens* must be very far from stable.[1] A majority of cells may contain more than one nucleus of each mating type, and in those cells with odd numbers of nuclei, more of one mating type than the other.

Since the fungus is situated in the phloem of the root it can easily enter branch roots (arising from the stele) and root-buds (arising from the stele and inner cortex). Both may be invaded by the fungus even before they emerge from the outer cortex of the parent root.

The systemic mycelium of shoots. The ability of the fungus to cause systemic infection of thistle shoots is apparently bound up with its ability to keep pace with the growing-point. Its growth rate in shoots is much higher than in other parts of the plant, e.g. the same mycelium which had grown only 2 cm. down the main root in 11 weeks had in the same time produced a systemic infection in a side shoot 20 cm. high. In all systemically infected shoots, hyphae may be detected within a few millimetres of the apex. In the older parts of the shoot the fungus is seen mainly in the stelar regions, both in the phloem and the interfascicular parenchyma. If infection is heavy, mycelium may be found throughout the tissues of the host.

Text-fig. 4. Multinucleate hyphae in the root xylem of the host.

The mycelium in uredosoral shoots is presumably heterokaryotic for mating type and is composed of binucleate cells, as one would expect. The surprising discovery is that the spermogonial mycelium which one might expect to be uninucleate since it is monokaryotic for mating type is also predominantly binucleate. For this reason the spermogonial mycelium will be described as homokaryotic for mating type.

The leaves of a considerable number of shoots, all of which had been kept unspermatized for some weeks, were examined by the hydrolysis-squash technique and also by microtome sections. In one spermogonial shoot, 9 uninucleate, 153 binucleate, and 87 trinucleate cells were counted; in another, 7 cells were uninucleate and 87 binucleate. In many of the squashes a number of adjacent cells (ranging from 3 to 10 in one hypha) could be scored. In 24 such hyphae all but 2 cells were binucleate. The exceptional ones were 3–1, obviously resulting from the unequal distribution of nuclei after division. In preparations of mycelium in stems the individual cells of the hyphae were usually so long that it was difficult to pick out the end walls. Counts made on hyphae in pith and cortex gave 7 uninucleate, 73 binucleate, and 3 trinucleate cells.

[1] For this reason the uredo mycelium is better referred to as heterokaryotic, signifying heterokaryotic for mating type, since this is the only character here considered.

The binucleate condition of the spermogonial mycelium persists as far as the base of the individual spermogonium. Within the spermogonium the spermatiophores, spermatia, and paraphyses are all uninucleate. Flexuous hyphae could be seen only on whole mounts of leaves and so very little internal structure could be made out. The development of the spermogonium was followed very closely to discover when the uninucleate condition arises. The smallness of the cells and the closely tangled plexus that is early formed precluded any attempt to follow nuclear changes in any particular hypha. Squash preparations, too, failed in this respect, since the cells of the constituent hyphae break apart under pressure. Sections do indicate, however, that the initial plexus is mainly binucleate (Text-fig. 5). Sections of older spermogonia show that some of the binucleate hyphae entering the base of the spermogonium give off uninucleate branches. It is probable that the uninucleate, fertile tissue is derived from such branches.

When spermogonial shoots were established from cuttings and kept isolated from other shoots, the spermogonial mycelium was found to grow out of the shoot into the new roots and infect new root-buds as they arose. Although the majority of the cells of this homokaryotic root mycelium were binucleate, there were a large number of trinucleate ones (Table VI).

TABLE VI. *The Nuclear Condition of Homokaryotic Root Mycelium*

Number of nuclei per cell	1	2	3	4	5	6
Number of cells	5	107	67	16	4	1

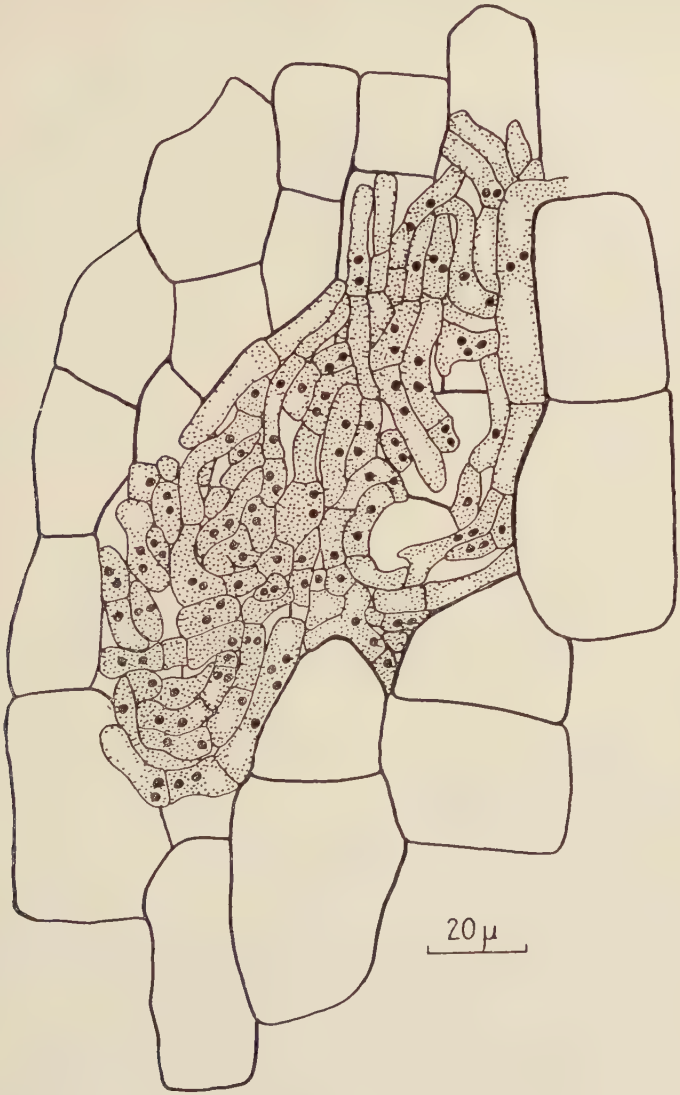
These figures should be compared with those given in Table V for the (presumably) heterokaryotic root infection derived from the uredo mycelium which also shows a tendency towards the multinucleate condition.

The mycelium developed after infection by basidiospores was also examined cytologically. Again, in every case, the hyphae were wholly binucleate. It must be noted, however, that the way in which the basidiospore infections were produced did not preclude the possibility of spermatizations having occurred prior to examination.

Cytology of the spermatized mycelium. Spermatization is generally supposed to occur through the union of spermatia of one mating type with flexuous hyphae of the other. Some controversy has centred round the behaviour of the spermatia. Many workers (Cornu, 1876; Plowright, 1889; Allen, 1930, 1932, 1934*a* and *b*; Lamb, 1935) claimed that they are able to germinate, either by a process of yeast-like budding or by producing elongated germ-tubes. In most recent work the feeling has grown that the budding of spermatia seen by earlier workers was nothing more than the budding of yeasts. This belief is substantiated by observations on *P. suaveolens*.

The spermatia of *P. suaveolens* are more or less oval with an average length of 0·5 μ and a width of 0·25 μ, comparable both in size and shape with the yeasts that occur naturally on thistle leaves and not easily distinguishable from them in unstained preparations. By using a Gram staining method, the

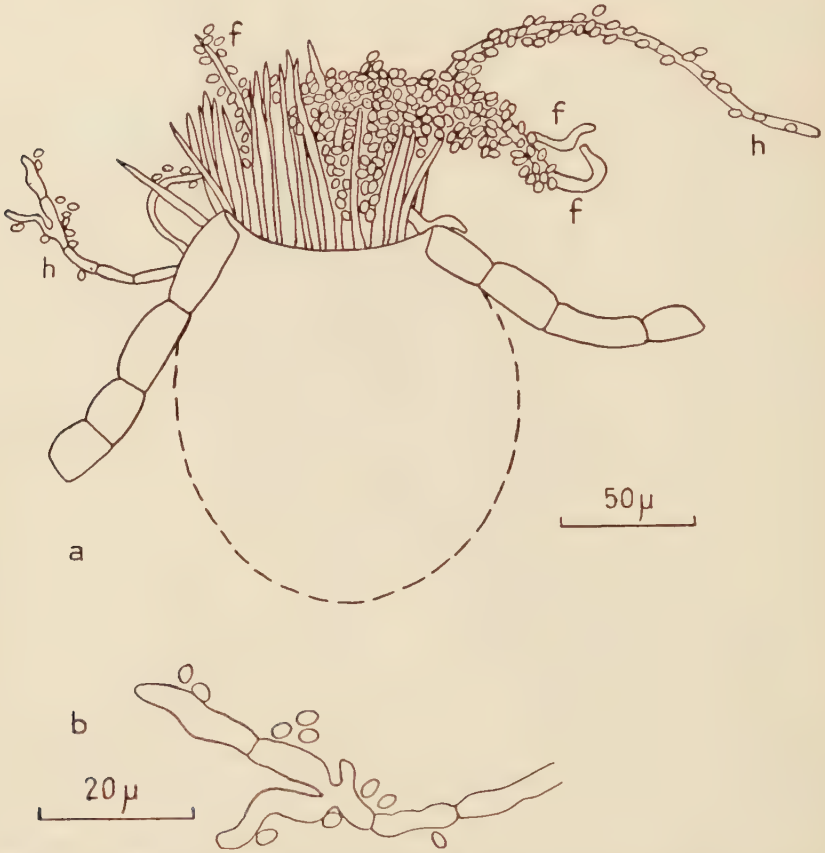
yeasts which stain a purplish black could be readily differentiated from the spermatia which stain a rosy pink. After a suspension of spermatia in 10 per



TEXT-FIG. 5. Section through the primordium of a spermogonium.

cent. glucose had been incubated for 24 hours, a number of cells were found to be budding off daughter-cells. All such cells stained purplish black by the Gram stain and were therefore yeasts. After 36 hours the spermatia appeared completely moribund and took up the stain only faintly, whereas the yeasts were increasing in number and actively budding. After 3 days a typical spermatization experiment was carried out with the suspension, which was

inoculated on to 15 leaves situated on 6 plants. In no case did primary uredospores develop, indicating that the spermatia were dead. Thousands of spermatia were examined by various staining methods, but none were ever seen to produce germ-tubes.



TEXT-FIG. 6. Whole mount of a spermogonium. *a*, whole spermogonium; *b*, detail of hypha at left of spermogonium; *f*, flexuous hyphae; *h*, septate, branched hyphae of other fungi growing in the spermogonial exudate.

Flexuous hyphae were difficult to find by the usual techniques. The long, branched, very slender, septate hyphae often seen were found to be hyphae of other fungi, e.g. conidiophores of *Penicillium*. Buller's whole mount method (Buller, 1950), however, revealed 4 to 6 flexuous hyphae projecting beyond the paraphyses of the spermogonia (Text-fig. 6). No spermatia were found adhering to flexuous hyphae on unspermatized leaves, whereas several were found on hyphae on plants growing in the field. It was also observed that the production of flexuous hyphae was restricted to a short period in the life of the spermogonium, when it was fully mature and actively exuding spermatia. Experimentally, too, this was the period when spermatization

occurred most readily, suggesting that the flexuous hyphae were concerned. Since these hyphae could not be seen in preserved material, no cytological investigations could be made into the union between hyphae and spermatia.

No uredosoral primordia were found prior to spermatization. The sorus was a completely new development, appearing 8 to 10 days after spermatization. Both in development and construction it closely resembled the secondary uredosorus. There was no evidence of cell fusion at its base. Difficulties were encountered when attempts were made to work out details of the spermatization process. The flexuous hyphae presumably connect with the plexus at the base of the spermogonium. The cells here were too small and contorted for their nuclear content to be examined. Outside the spermogonium the cells were already binucleate. Several spermatized shoots were kept until they were producing nothing but uredosori. In these, too, the mycelium was binucleate and could not be distinguished from that prior to spermatization. The assumption is made that the nucleus introduced from the spermatium migrates through the mycelium until it reaches a hyphal tip and there the production of the uredosorus begins at once.

Discussion

Inoculation experiments have confirmed Buller and Brown's finding (Buller, 1950) that the spermogonial mycelium of *Puccinia suaveolens* may arise in the normal way by infection with basidiospores. Teleutospore germination, however, is so erratic that the amount of infection caused by basidiospores in nature is probably negligible. Most of the spermogonial mycelium appears to arise from the heterokaryotic mycelium produced by infection with uredospores. Most interest centres round the way in which the homokaryotic spermogonial mycelium may arise from a heterokaryotic uredo mycelium, that is the process called 'de-diploidization' by Buller and equivalent to a somatic segregation of mating type.

Buller and Brown (1941) first showed, and the experiments here described are in agreement, that the spermogonial mycelium of this fungus is heterothallic. There are two mating types, occurring in approximately even numbers. Buller and Brown (1943) believed that de-diploidization occurred in the young root-buds and that homokaryotic branches invaded the leaf rudiments. On this reasoning both mating types should be represented in different leaves of the same shoot. Buller (1950) appreciated this and claimed that the shoot on which they had carried out their spermatization experiment was exceptional in that all the leaves bore spermogonia of the same mating type and hence de-diploidization must have occurred at the shoot base. It is most probable that Buller was confused by the fact that he had seen binucleate mycelium in the stem of spermogonial shoots. The experiments here described show that the mycelium throughout the spermogonial shoots is homokaryotic for mating type, thus suggesting that segregation must generally take place at the base of the shoot. The many cases in which several spermogonial shoots arising at about the same time bear mycelium of the same

mating type suggest that segregation may even take place in the root and that the homokaryotic mycelium may invade a number of adjacent shoots. Infected shoots do show a tendency to arise in clusters like witch's brooms. It is unlikely that segregation takes place nearer the original point of infection since the first infected shoots of many plants bear uredosori only (Table II).

When this work was begun it was hoped that cytological investigation of the root and shoot mycelium would show where segregation of mating type takes place. The discovery of the binucleate condition of both the uredosoral and spermogonial mycelium before and after spermatization and the irregular condition of the root mycelium made this impossible. Other workers have been puzzled by the presence of binucleate cells in the spermogonial mycelium. Olive (1913) suggested that both the uninucleate and binucleate mycelia were present in all systemically infected shoots and that there was competition between them. His account appears to be an ingenious attempt to explain the phenomenon of heterothallism in this fungus. Kursanov (1922) later showed that the mycelium producing spermogonia was in fact binucleate, uninucleate cells appearing only in the vicinity of the spermogonium. Buller (1950), too, saw only binucleate cells in the stem mycelium, and this appears to have led to his postulate that de-diploidization must take place at the base of each leaf.

It is possible that the mycelium described in all the above accounts had already been spermatized and made heterokaryotic before it was examined. In the present investigation all the shoots examined had been carefully kept unspermatized: in them, too, the mycelium was predominantly binucleate, uninucleate cells appearing only in the immediate vicinity of the spermogonia. It is inconceivable that segregation of the same mating type occurs at the base of each of the very numerous spermogonia on each shoot. One is thus forced to the conclusion that segregation takes place farther back, at least at the base of the shoot, and that the paired nuclei in the unspermatized mycelium are both of the same mating type. It is unfortunate that no means of tagging the nuclei with a mutant character could be devised.

As an alternative to de-diploidization, the possibility was considered that the uredospores, instead of being heterokaryotic for mating type, might bear different combinations of nuclei. Thus, if the two mating types are represented by the letters *A* and *a*, then the uredospores might have the following nuclear constitutions: *AA*, *Aa*, or *aa*. A systemic infection arising from an *AA* spore would then produce spermatia of only one mating type, *aa* spermatia of the opposite mating type, and *Aa* uredospores only. In order to investigate this possibility, 100 single uredospore cultures were established on seedling thistles and carefully protected from further infection. Thirty of these infections subsequently became systemic. The types of infection were very similar to those arising from mass inoculations. Although some plants did bear only one type of infected shoot (12 with only spermogonial ones and 5 with uredosoral), there were also a large number of plants (13 in all) which bore several shoots of both types. For these 13 plants one would still have to postulate some form of de-diploidization or diploidization to account for the

production of spermogonial mycelium from uredo or vice versa. Therefore heterogeneity among the uredospores could not be the only solution to the problem.

A report of a binucleate condition in the homokaryotic mycelium similar to that in *P. suaveolens* has been made by Brodie (1950). In all the examples of unilateral diploidization observed by him in *Cyathus stercoreus* he found that the acceptor mycelium was binucleate. Where the binucleate condition arose was unknown, but it could be produced artificially by combining two different haploids of the same mating type. This evidence of the pairing of nuclei of like mating type invites further consideration of the question of conjugate division in the rusts, about which Dodge (1942) has already cast some doubt. In *Puccinia suaveolens*, too, the origin of the binucleate condition in the homokaryotic mycelium is unknown. The evidence indicates that as the mycelium passes out of the roots into the shoots it changes from the multinucleate condition to the binucleate. If the two nuclei that pass into a hyphal branch at the shoot base happen to be of the same mating type, then the mycelium will bear spermogonia; if of opposite mating type, the shoot will bear uredosori. Once the mycelium enters a root-bud it grows away very rapidly, takes possession of the shoot, and excludes all others.

Summary

The autoecious rust *Puccinia suaveolens* which attacks the creeping thistle *Cirsium arvense* shows two types of infection, an isolated mycelium bearing uredosori with teleutospores, and a systemic one bearing either spermogonia or uredosori. The isolated mycelium is produced by inoculation with uredospores. This mycelium later grows out of the inoculated leaves into the roots and invades the root-buds as they arise. In some buds the mycelium appears unchanged and produces uredosori; in others it has undergone a somatic segregation of mating type (Buller's de-diploidization) and produces spermogonia. Some of the spermogonial mycelium may also be derived from infection by basidiospores, but germination of teleutospores is so erratic that only a small amount appears to arise thus.

Puccinia suaveolens is heterothallic and there are two mating types. All the spermogonial mycelium in one shoot is of the same mating type. There is also a tendency for all the spermogonial shoots on one plant to bear spermogonia of the same mating type. It follows that segregation of mating type must occur at least at the base of a shoot or even farther back in the root.

The nuclear behaviour of the fungus is irregular. Much of the mycelium produced by infection with binucleate uredospores is trinucleate. There are from 4 to 6 nuclei in most hyphal tips. The systemic mycelium in shoots, on the other hand, is all binucleate whether it produces spermogonia or uredosori. No cytological differences can be observed in the spermogonial mycelium before and after spermatization. In the roots both homo- and heterokaryotic mycelium tends to be multinucleate. Because of these peculiarities, neither segregation of mating type nor spermatization can be

observed directly. It is believed that as the mycelium invades a root-bud it changes from the multinucleate to the binucleate condition. If the paired nuclei happen to be of the same mating type, the shoot produces spermogonia; if of opposite mating type, uredosori.

Acknowledgements

I am greatly indebted to the late Professor Brooks for suggesting this problem and for his helpful advice and criticism throughout the course of the work. I should also like to thank Dr. N. F. Robertson for his help and Mr. F. T. N. Elborn for the photograph of Pl. XXXIII, Fig. 1.

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FIG. 1. Uredosori produced on a spermogonial shoot 10 days after spermatization. The leaves are numbered from the base to the apex of the shoot. Leaves 2, 3, and 4 were spermatized. The greatest number of uredosori appear on the youngest leaf treated. Uredosori are also beginning to appear on leaves 5 and 6, particularly along the mid-ribs.

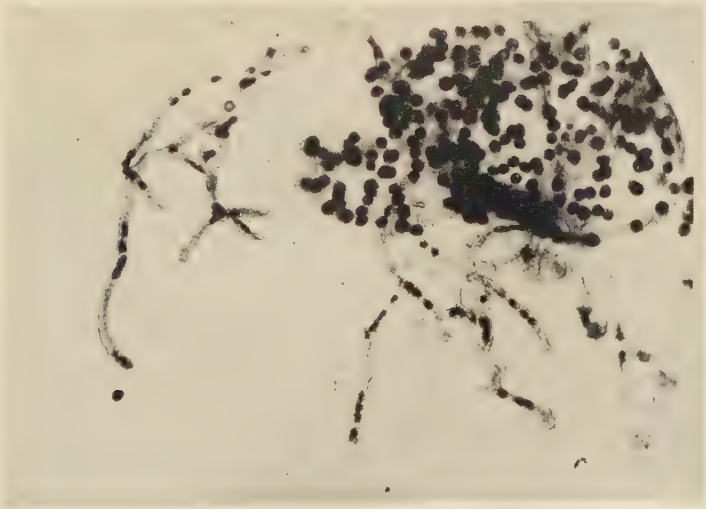

FIG. 2. Squash preparation of the mycelium produced in a leaf after inoculation with uredospores. Most of the hyphal tips are multinucleate.

An Examination of the Factors affecting Variability in the Growth of the Mesocotyl and Coleoptile of Etiolated *Avena* Seedlings

BY

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Abstract

Experiments were carried out on the effect of rate of air-flow and of humidity on the growth of *Avena* seedlings. The experimental methods used are described in some detail.

The effect of air-flow on mesocotyl growth was considerable when unsaturated air was used, but with saturated air growth was independent of air-flow. The effect must therefore be due to variation in transpiration rate. A 10 per cent. variation in relative humidity, at a constant rate of flow, caused a 30 per cent. variation in mesocotyl length. The coleoptiles showed less pronounced effects, but even in saturated air variability was not eliminated.

Under the conditions used a difference of 2·3 mm. in mesocotyl length (per 50 plants), i.e. 8 per cent., was significant at $P = 0{\cdot}05$; for the coleoptiles a difference of 1 mm. (8 per cent.) was significant at this level.

Introduction

ONE of the many difficulties in the field of hormone research is that of the variability of the *Avena* seedling, which manifests itself not only in the early growth of the plants but also—in the standard assay method—in differing responses to the same concentration of hormone on different occasions.

The literature on the subject has been reviewed by Moevus (1949), and this together with further contributions by Larsen (1948) and Bentley (1950) indicates the effort that has been made to overcome the difficulty in relation to the coleoptile. No comparable work has been carried out on the behaviour of the mesocotyl; the only relevant information is that published by Kempton (1943), who stated: 'Lack of agreement between repeated experiments and excessive variability of sub-groups within experiments compelled increasing refinements in control of conditions of growth and led eventually to a study of the effect of the solution upon developing seedlings.'

This quotation accurately describes the situation encountered at the beginning of this work, but the present experiments indicated that humidity was the factor largely responsible for the variability.

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Preliminary work had been started on the effects of carbon dioxide on the growth of etiolated seedlings (Mer and Richards, 1950), and the experience there gained showed that the sources of variability would need to be eliminated before further work could advantageously be undertaken.

Materials and Methods

Avena sativa var. 'Victory' was used for all experiments. The seed was dehusked and planted without preliminary soaking in glass tubes ($2\frac{1}{2}$ in. long and 6 mm. internal diameter) in which were inserted two unfolded slips of filter-paper, each $2\frac{1}{4}$ in. long and $\frac{1}{4}$ in. wide. The grain was held at the top of the seed-holder between these paper strips. Three seed-holders were placed in a specimen tube containing a little tap-water, which reached the seed by capillary action. This technique differs slightly from that previously described (Mer, 1951), employing a single slip of filter-paper doubled over lengthwise to hold the seed in position. Germination was found to be very uneven using this earlier method, due to the presence of a film of water around the seed carried up to it by the filter-paper. With the introduction of the modification noted here the distance between the seed and the water-meniscus in the specimen tube was increased to 2 in. (formerly it had been 1 in. or less) and the variation in germination was thereby reduced.

The plants were grown in an incubator, provided with upper and lower shelves, maintained at a temperature of 24° C.$\pm$0·2° C.; undesirable temperature gradients were minimized by incorporating a fan to stir the air vigorously.

Six sets of fifty-seven plants were grown in each of two light-tight containers (occupying the two incubator shelves), provided with the necessary inlet and outlet ports for the gas streams with which the plants were continuously aerated. Over each set of fifty-seven plants an inverted litre beaker was placed, which was lined with damp blotting-paper to maintain a humid atmosphere. Upper and lower containers, therefore, each held two rows of three beakers.

The growth of *Avena* seedlings is known to be adversely affected by air contaminated with coal-gas (Crocker, 1948); consequently the air pumped over the plants was drawn from outside the laboratory. The air was filtered, moistened by passage through a water-column, and supplied at a flow-rate of either 60 or 120 litres/hour to each container, i.e. 10 or 20 litres/hour to each beaker, as required in the different experiments.

With the water-column at laboratory temperature 'moist' air entered the incubator, but when saturated air was required the column was warmed to 33–35° C., and the air, on passing into the incubator, deposited the excess moisture, which was trapped in tubes provided for the purpose. Inside the incubator the gas passed through tubing to allow the necessary temperature exchanges to take place and entered the containers, in which the stream divided and passed into each of the six beakers. The air descended over the seedlings and then escaped into the atmosphere.

Experimental Results

The extent of the variability among the sets of plants treated similarly on one occasion is shown by the measurements in Table I. In this and the following tables the data are set out to correspond with the positions occupied in plan view by the individual sets of plants within the containers. Analysis of variance indicates that the differences between container- and beaker-means are highly significant.

Table I

Expt. 1. Length (mm.) of Plants grown for 3 Days in 'Moist' Air. Flow-rate 60 litres/hour. No. of replicates in brackets

	Plan view of upper container.			Plan view of lower container.		
Mesocotyls	22·7	25·2	25·5	20·2	21·2	18·5
Coleoptiles	12·4	13·6	13·4	12·0	11·7	11·9
	(54)	(53)	(54)	(53)	(54)	(51)
Mesocotyls	26·2	25·1	25·1	19·8	18·2	22·3
Coleoptiles	13·6	14·1	15·2	12·8	13·1	11·7
	(50)	(50)	(55)	(51)	(53)	(51)

Analysis of Variance

	DF	Mesocotyls. Mean sq.	Mesocotyls. F. ratio.	Coleoptiles. Mean sq.	Coleoptiles. F. ratio.
Containers	1	3831·29	124·51**	314·80	37·39**
Beakers	5	99·04	3·22**	38·08	4·52**
Interaction	5	103·78	3·37**	41·61	4·94**
Error	617	30·77	—	8·42	—
Total	628	37·94	—	9·41	—

Significance: * $P = 0{\cdot}05$; ** $P = 0{\cdot}01$.

1. *The effect of aeration*

i. In preliminary experiments employing *saturated* air flowing at 60 litres/hour the inlet tubes to some of the beakers became blocked by water condensing from the air stream. It was noted that the mesocotyls of the plants in these beakers, having been exposed for some time to still air, were conspicuously long and grew either horizontally or obliquely upward, while the coleoptiles were short and showed strong upward curvatures. The adequately aerated plants were shorter with upright plumules.

The effect of air-flow was therefore investigated at three levels with two beakers per treatment in each container. The treatments were: (*a*) still air: the inlet tubes to the appropriate beakers were closed; (*b*) low flow-rate: short lengths of capillary tubing were included in the leads to the beakers; and (*c*) high flow-rate: the rest of the air stream was divided between the remaining two beakers. Moist, but unsaturated, air was used and because the

flow-rate per container was 120 litres/hour, that through the beakers in treatment *c* was well in excess of 20 litres/hour.

The result of one experiment is recorded in Table II. It will be seen that there was a progressive lengthening of the mesocotyl with decreasing rate of flow, while the coleoptiles were shortest in still air and similar in the other two treatments. The analysis of variance shows further that the variability attributable to containers reached the 1 per cent. level of significance.

TABLE II

Expt. 2. The Effect of Air Flow-rate on the Length of Seedlings (mm.) grown for 3 Days in Moist Air. No. of replicates in brackets

	Upper container.			Lower container.		
Mesocotyls . .	20·6	28·6	25·9	23·7	30·0	27·6
Coleoptiles . .	12·0	8·5	12·6	14·0	9·3	13·3
Treatment . .	*c*	*a*	*b*	*c*	*a*	*b*
	(49)	(52)	(52)	(49)	(52)	(53)
Mesocotyls . .	28·2	24·5	20·4	28·9	26·6	21·8
Coleoptiles . .	8·7	13·6	13·5	8·1	13·5	13·3
Treatment . .	*a*	*b*	*c*	*a*	*b*	*c*
	(55)	(49)	(47)	(54)	(53)	(45)

a, still air; *b*, low flow-rate; *c*, high flow-rate.

Analysis of Variance

		Mesocotyls.		Coleoptiles.	
	DF	Mean sq.	F. ratio.	Mean sq.	F. ratio.
Containers .	1	422·23	17·79**	29·33	6·05**
Air-flow . .	2	2721·82	114·65**	1437·67	296·43**
Interaction .	2	13·90	n.s.	8·58	n.s.
Error . .	604	23·74	—	4·85	—
Total . .	609	33·23	—	9·61	—

Significance: * $P = 0{\cdot}05$; ** $P = 0{\cdot}01$.

In a second experiment the same treatments were used but allotted to different beakers. The measurements agreed with those recorded in Table II and varied similarly with treatment, indicating that differences in length were associated much more with rate of air-flow than with the particular beakers in which the plants were grown.

ii. When the seed was planted as already described the lower end of the seed-holder dipped into the water-supply so that there was no flow of air past the seed and root. Plants were therefore grown in seed-holders, which were bound together in groups of fifty-seven with three glass rods included to act as a tripod support, thus raising the holders well above the water-level. The specimen tubes were temporarily dispensed with and the seeds supplied with water by pushing the filter-papers down until they projected about 1½ in. below the rims of the seed-holders, and adjusting the tripod legs so that the protruding filter-papers dipped into a dish of water. The plumule normally

grew out into the air, but in this particular experiment in which the seeds were lowered into the tubes with the filter-paper, it grew completely within the seed-holder.

The results of experiments employing the upper container on two occasions are recorded in Table III. They show that seedling growth in the 'raised' seed-holders was much more uniform than with the normal method; mesocotyls were consistently longer and coleoptiles consistently shorter. The analysis of variance shows that in the 'raised' plants the variance in length of mesocotyls associated with both occasion and beakers did not reach significance and the results were therefore reproducible. The coleoptiles were less uniform, for the variance associated with beakers reached the 1 per cent. level of significance.

TABLE III

Expts. 3 and 4. The Effect of Method of Planting on the Length (mm.) of Seedlings grown for 3 Days in Moist Air. Flow-rate 120 litres/hour. No. of replicates in brackets

	Expt. 3.			Expt. 4.		
Mesocotyls . .	26·4	24·8	26·1	21·5	27·2	23·1
Coleoptiles . .	9·4	13·6	9·2	14·1	10·3	13·1
Treatment . .	*r*	*n*	*r*	*n*	*r*	*n*
	(54)	(52)	(51)	(49)	(46)	(51)
Mesocotyls . .	21·9	27·3	25·4	27·8	21·2	25·6
Coleoptiles . .	13·9	10·7	13·3	10·0	13·3	9·9
Treatment . .	*n*	*r*	*n*	*r*	*n*	*r*
	(49)	(50)	(48)	(47)	(46)	(47)

n = normal planting; *r* = 'raised' planting.

Analysis of Variance of the 'Raised' Plants

		Mesocotyls.		Coleoptiles.	
	DF	Mean sq.	F. ratio.	Mean sq.	F. ratio.
Occasion . .	1	4·18	n.s.	7·67	n.s.
Beakers . .	4	27·34	n.s.	17·09	4·94**
Error . .	289	32·52	—	3·46	—
Total . .	294	33·51	—	3·66	—

Significance: * $P = 0.05$; ** $P = 0.01$.

These experiments indicated that differences in seedling growth might be induced by variation in aeration, and as a result an examination was made of the air distribution between the several beakers. Although the tubing within the containers had been arranged symmetrically, air-flow to individual beakers was found to vary considerably, and by altering the positions of the beakers with their inlet tubes the whole pattern of flow changed.

The rate of flow in the individual beakers was roughly determined by absorbing the water-vapour in the air stream in sulphuric acid and weighing. It was noted that the beakers in which the longer mesocotyls had at first been found were also those with the least air-flow. The beakers were

rearranged to secure more uniform air-flow and the experiment repeated. The results for one container are shown in Table IV and indicate that with more uniform air-flow the plants were less variable.

TABLE IV

Expt. 5. Length (mm.) of Plants grown for 3 Days in Moist Air flowing at 60 litres/hour after Rearrangement of Beakers

Mesocotyls	21·3	21·9	21·8
Coleoptiles	11·8	11·0	10·6
	(46)	(51)	(50)
Mesocotyls	21·5	21·2	22·3
Coleoptiles	11·2	10·6	11·2
	(47)	(41)	(52)

Analysis showed that the variance among beakers for both mesocotyls and coleoptiles did not now reach the level of significance.

2. *The effect of humidity*

The effect of humidity on seedling growth was also investigated at three levels: (*a*) low humidity: the water column was omitted and air was led directly into the incubator; (*b*) medium humidity: using moist air as in the previous experiments; and (*c*) high humidity: the air stream was saturated by previous passage through water in the column heated to 33–35° C. The experiments were carried out consecutively. Normal planting technique was used and the rate of air-flow was 120 litres/hour to each container. The data are recorded in Table V. The effect of humidity, which included variability due to occasion, was calculated by pooling the data. It was highly significant ($F = 281{\cdot}45$).

The results show that the length of the mesocotyl increased with increasing humidity and that the excessive variability between containers in the low humidity treatment ($F = 11{\cdot}98$) disappeared at the higher humidity levels. Uniformity between beakers was found throughout. The coleoptiles similarly increased in length with increasing humidity but to a lesser extent than did the mesocotyls.

TABLE V

Length (mm.) of Seedlings grown for 3 Days in Air of Different Humidity. Flow-rate 120 litres/hour to each container. No. of replicates in brackets

Expt. 6. Low Humidity

	Upper container.			Lower container.		
Mesocotyls	21·5	21·8	20·5	23·2	21·8	21·3
Coleoptiles	11·2	12·5	12·2	12·3	12·0	11·8
	(50)	(47)	(45)	(51)	(51)	(50)
Mesocotyls	20·6	21·7	22·3	23·6	23·3	22·8
Coleoptiles	11·2	11·7	12·4	11·7	12·1	12·1
	(45)	(50)	(53)	(52)	(50)	(45)

TABLE V (*continued*)

Analysis of Variance

	DF	Mesocotyls. Mean sq.	Mesocotyls. F. ratio.	Coleoptiles. Mean sq.	Coleoptiles. F. ratio.
Containers	1	287·30	11·98**	2·93	n.s.
Beakers	5	35·31	n.s.	9·03	2·13*
Interaction	5	39·39	n.s.	9·93	2·34**
Error	577	23·99	—	4·24	—
Total	588	24·66	—	4·33	—

Significance: *P=0·05; **P=0·01.

Expt. 7. Medium Humidity

	Upper container.			Lower container.		
Mesocotyls	26·6	27·2	27·2	27·8	27·3	28·0
Coleoptiles	13·1	13·5	12·8	13·0	12·2	13·1
	(51)	(50)	(51)	(48)	(51)	(54)
Mesocotyls	26·9	26·3	27·8	27·1	27·4	25·2
Coleoptiles	12·4	12·8	13·4	12·5	12·2	12·0
	(53)	(52)	(53)	(52)	(49)	(51)

Analysis of Variance

	DF	Mesocotyls. Mean sq.	Mesocotyls. F. ratio.	Coleoptiles. Mean sq.	Coleoptiles. F. ratio.
Containers	1	2·26	n.s.	38·20	7·04**
Beakers	5	13·66	n.s.	6·39	n.s.
Interaction	5	50·74	n.s.	14·20	2·62**
Error	603	36·60	—	5·43	—
Total	614	36·47	—	5·56	—

Significance: * $P = 0·05$; ** $P = 0·01$.

Expt. 8. High Humidity

	Upper container.			Lower container.		
Mesocotyls	29·9	29·2	29·7	29·6	29·2	28·8
Coleoptiles	14·0	12·6	13·8	12·6	12·7	12·7
	(53)	(53)	(54)	(53)	(51)	(54)
Mesocotyls	29·2	28·6	30·4	28·1	29·5	28·9
Coleoptiles	12·7	12·8	13·2	12·9	12·2	12·4
	(55)	(53)	(55)	(52)	(53)	(53)

Significance: * $P = 0·05$; ** $P = 0·01$.

Analysis of Variance

	DF	Mesocotyls. Mean sq.	Mesocotyls. F. ratio.	Coleoptiles. Mean sq.	Coleoptiles. F. ratio.
Containers	1	41·71	n.s.	48·96	8·13**
Beakers	5	18·17	n.s.	12·16	2·02*
Interaction	5	19·21	n.s.	11·09	n.s.
Error	627	29·63	—	6·02	—
Total	638	29·48	—	6·18	—

Significance: * $P = 0·05$; ** $P = 0·01$.

So far as the mesocotyls are concerned the data show that variability can be reduced below statistically significant levels by increasing the humidity of the air stream; this holds true for variances associated with both containers and beakers. The reason for the high *F* ratio in the low humidity treatment is not at all clear as every effort was made to equalize conditions between the two containers. In the contemplated experiments on the effect of carbon dioxide on mesocotyl extension it will be necessary to arrange controls and treated plants in separate containers and differences between them would therefore be confounded with treatment effects. These experiments showed, however, that container differences failed to appear when saturated air was used.

The coleoptile data show that the variance associated with containers rose with increasing humidity. Again, no explanation can be offered. In expts. 6 and 8 beaker variance reached significance; in expts. 6 and 7 the interaction variance did likewise. The elimination of variability in coleoptile length would thus appear to be inherently more difficult.

Because the length of the mesocotyl was maximal in saturated air it was used for all subsequent experiments. A re-examination was made of the effect of air-flow at this humidity level by growing plants in saturated air flowing at 60 litres/hour. The measurements are recorded in Table VI, and it will be seen that they compare with those of expt. 8, in which the flow-rate was 120 litres/hour.

TABLE VI

Expt. 9. Length (mm.) of Seedlings grown for 3 Days in Saturated Air flowing at 60 litres/hour

	Upper container.			Lower container.		
Mesocotyls . .	28·3	29·3	28·8	30·4	29·0	28·4
Coleoptiles . .	13·2	13·1	12·4	13·7	13·6	12·0
	(49)	(47)	(48)	(45)	(45)	(50)
Mesocotyls . .	28·9	29·4	27·4	29·0	27·4	28·8
Coleoptiles . .	13·4	13·5	13·0	12·6	12·5	11·9
	(43)	(46)	(49)	(49)	(46)	(47)

The statistical analysis of these data (Table VII) shows that the effect of air-flow, although including variability due to occasion, was not significant.

It will also be seen that the mesocotyl data were again more uniform than those of the coleoptiles.

Trials have since been carried out under these conditions (saturated air, 60 litres/hour), and from the data so accumulated (6 expts.) those in Table VIII are quoted to illustrate the extremes of variation so far observed between occasions.

The mean mesocotyl lengths in expts. 8, 9, 10, and 11 are respectively 29·3, 28·8, 27·4, and 29·7 mm. From the combined data a pooled error variance of 31·22 has been extracted, so that a difference between these means of

TABLE VII

Statistical Analysis of Expts. 8 and 9

	DF	Mesocotyls. Mean sq.	Mesocotyls. F. ratio.	Coleoptiles. Mean sq.	Coleoptiles. F. ratio.
Air-flow	1	75·48	n.s.	0·01	n.s.
Containers	1	11·56	n.s.	68·41	11·26**
Beakers	5	18·17	n.s.	14·30	2·36*
Beakers × Air-flow	5	21·98	n.s.	19·92	3·28**
Beakers × Containers	5	14·07	n.s.	9·80	n.s.
Air-flow × Containers	1	34·06	n.s.	2·00	n.s.
Triple interaction	5	55·13	n.s.	13·65	2·25**
Error	1,179	32·24	—	6·07	—
Total	1,202	32·18	—	6·26	—

Significance: * $P = 0.05$; ** $P = 0.01$.

TABLE VIII

Expts. 10 and 11. Length (mm.) of Plants grown for 3 Days, on Different Occasions, in Saturated Air flowing at 60 litres/hour

	Upper container.			Lower container.		
Mesocotyls	27·0	28·1	28·4	26·9	27·3	26·8
Coleoptiles	12·0	13·8	13·2	13·4	12·6	13·1
	(40)	(49)	(49)	(46)	(43)	(40)
Mesocotyls	27·4	26·4	28·2	26·5	27·7	28·0
Coleoptiles	12·7	12·9	13·2	13·5	13·4	12·3
	(49)	(41)	(51)	(47)	(50)	(42)
Expt. 11						
Mesocotyls	29·6	31·0	28·6	30·3	29·7	28·9
Coleoptiles	12·6	12·4	12·6	11·8	12·4	12·0
	(47)	(46)	(42)	(51)	(48)	(41)
Mesocotyls	30·1	30·2	30·4	28·9	29·5	29·2
Coleoptiles	11·9	12·5	12·1	13·0	11·9	12·8
	(49)	(47)	(50)	(46)	(48)	(40)

0·8 mm. is required for significance at $P = 0.05$ (assuming 576 replicates). Within experiments 2·3 mm. difference between beaker means is necessary for significance at this level (assuming 50 replicates). The data show that while within experiments the growth of the mesocotyl was adequately uniform it was not consistently so between occasions.

The mean coleoptile lengths in the four experiments were 12·9, 12·9, 13·0, and 12·3. With a pooled error variance of 5·30 a difference between them of 0·3 mm. only is required for significance at $P = 0.05$, while within experiments a difference of 1·0 mm. between beaker means is significant. The data show that the variability is beyond this limit.

DISCUSSION

The aeration effects recorded here may be explained in terms of humidity variation. The lengths of the mesocotyls in the several treatments in expt. 2

agree with those recorded in Table V; thus in still air the humidity would approach saturation and the mesocotyl lengths are similar to those in expts. 8–11 in which saturated air was employed. Again, when 'raised' seed-holders were used the plumule would grow in a very humid atmosphere and the measurements approach those recorded for the high humidity treatments.

It seems probable, therefore, that the effect of air-flow at low humidities was due to the exposure of the seedlings to differing conditions of transpiration. The variability encountered at the start of this work would then have arisen from the interaction between low humidity and unequal rates of air-flow through the beakers.

The effect of humidity at constant flow-rate was appreciable. The difference in relative humidity between expts. 6 and 8 was about 10 per cent. and it produced a change of 30 per cent. in mesocotyl length.

The use of saturated air thus masked the variability arising from the unequal distribution of air between the beakers as far, at least, as the mesocotyls were concerned. The persistent variability of the coleoptile seems to indicate that more stringent control of environmental conditions is necessary for its uniform growth, but no effort has been made to determine these conditions.

Acknowledgement

The writer wishes to record his indebtedness to Dr. F. J. Richards for his invaluable advice and critical discussion during the investigation. Mr. Douglas Rees, of the Statistics Dept., Rothamsted Experimental Station, assisted with many of the statistical analyses and gave useful advice on the design of experiments. Miss M. J. Foster helped with the preparation of the experiments and with the recording of the results. Thanks are also due to Professor F. G. Gregory, F.R.S., for his unfailing encouragement and support throughout the course of the work.

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Scottish Lower Carboniferous Plants: *Eristophyton waltoni* sp. nov. and *Endoxylon zonatum* (Kidston) Scott from Dunbartonshire

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With Plate XXXIV and six Figures in the Text

Abstract

Two petrified stems are described. One, *Eristophyton waltoni*, which only differs from *E. beinertianum* (Goeppert) Zalessky, in details, provides new information about the leaf trace and its occlusion after leaf fall. The other, identified as *Endoxylon zonatum* (Kidston) Scott, differs from the Type chiefly in showing no growth rings; it indicates the range of variation in the genus and an emended generic diagnosis is given.

Both genera, formerly included in the Calamopityaceae, are now tentatively referred to the Cordaitales.

Introduction

THE new specimens described here were collected by Professor J. Walton and kindly entrusted to me for investigation. Both were obtained from natural exposures in the Kilpatrick Hills, Dunbartonshire. *Eristophyton waltoni* came from a small stream on the south side of Loch Humphrey Burn and *Endoxylon zonatum* from Glenarbuck, near Bowling. They both occurred in sediments containing volcanic ash lying below the Clyde Plateau Lavas and referred to the Cementstone Group of the Calciferous Sandstone Series, Lower Carboniferous (see Walton, 1949, pp. 719, 732).

The specimen of *Eristophyton waltoni* is from a classic locality, and was found only a few feet above where the following stems were obtained:

Eristophyton fasciculare (Scott) Zalessky; Scott, 1902, 1918; *Calamopitys radiata* Scott and *Bilignea resinosa* Scott; see Scott, 1924.

The rock matrix is a volcanic ash redeposited by water and the stem of *E. waltoni* is mainly silicified, while that of *E. zonatum* is mainly calcified. Three rock sections of *E. waltoni* had been prepared by W. Hemingway and for the rest I prepared numerous Cellulose Peel sections of both stems.

The Genus *Eristophyton* Zalessky

The name *Eristophyton* was originally proposed by Zalessky (1911) to include two pycnoxylic species of the genus *Calamopitys* (*C. fascicularis* and *C. beinertiana*) which in his opinion did not show close resemblance to the Lyginopterideae or even in general with the Cycadofilices. Since Zalessky did not give a precise definition of his new genus and no subsequent writers

on the subject have yet done so, it is desirable to give a diagnosis of *Eristophyton* before proceeding to the description of the new species.

Diagnosis. Woody stems. Pith varying from small to rather large, continuous, purely parenchymatous, or partly parenchymatous with sclerotic tissue forming nests, but no medullary tracheides or xylem strands. Primary xylem consisting of the decurrent portions of leaf traces forming numerous strands close to or in contact with the secondary xylem, mesarch above, but much reduced in size and endarch or nearly so below. Leaf traces undivided in their inner parts, numerous and spirally arranged, with short internodes. Secondary xylem compact, sometimes showing growth rings, composed of narrow tracheides and small to medium-sized rays. Bordered pitting of the secondary tracheides chiefly on the radial walls, multiseriate, with horizontal or oblique pit-apertures. Rays parenchymatous, up to 5 cells wide but most commonly only 1 or 2 cells wide, and up to 50 cells but most commonly less than 30 cells high. Ray field, where known, with 2 to 10 large obliquely placed bordered pits. Tissues outside the secondary xylem, where known, consisting of a bark formed of successive layers of periderm.

Eristophyton waltoni sp. nov.

Diagnosis. Pith about 1 cm. wide containing large sclerotic nests, up to 3·0 mm. wide. Sclerotic nests sharply divided into inner and outer zones. Primary xylem strands about 10, very small, at most only 0·16 mm. wide where about to enter the secondary wood as leaf traces. Leaf traces in a close complex spiral. Secondary wood with some tangential pitting, a little xylem-parenchyma, and growth rings. Rays 1 to 5 cells wide and commonly 30 but up to 50 cells high.

Type specimen, Hunterian Museum, Pb. 2367 (and original slab of rock with fragments, Pb. 2368), University of Glasgow. The *type sections* are 423 to 451, Figured Slide Collection, Department of Botany, University of Glasgow.

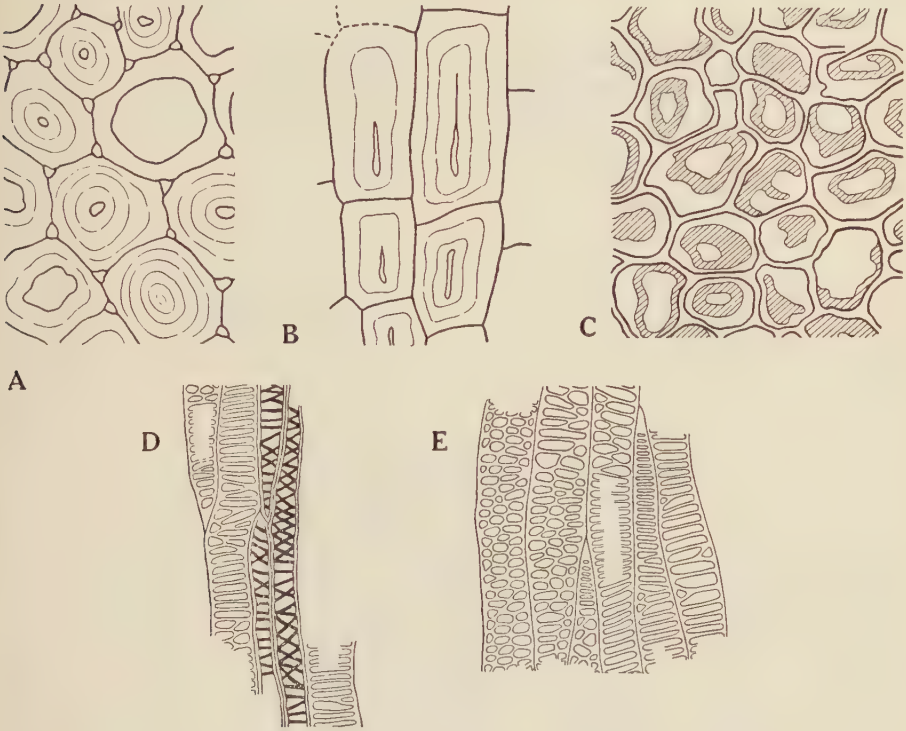
Description

The stem was 44 cm. long and up to 3.5 cm. wide. All the outer tissues are missing and the surface formed by the secondary wood is worn (Pl. XXXIV, Figs. 1 and 2, and Text-figs. 4, J and K).

The *medulla* is crushed and ill preserved, but the continuous part is a thin-walled parenchyma composed of uniform cells 50 μ to 80 μ in both length and breadth. The sclerotic nests are scattered irregularly; 10 to 50 may be seen in a single transverse section. Their shape in transverse section varies from round to very irregular and their size also varies greatly (Pl. XXXIV, Fig. 2). That shown in Pl. XXXIV, Fig. 3, is typical; it is just over 2 mm. wide and shows the central core of moderately thick-walled cells surrounded by a zone of thicker-walled elongated cells which cease abruptly at the outside. In longitudinal section they are similar. Details of the cells are shown in Text-fig. 1, A to C.

There are no tracheides at all in the medulla, and no vascular strands apart from those constituting the primary xylem system at the periphery.

The *primary wood* is merely the downward extension of the leaf traces; there is no purely cauline wood. As seen in transverse section it consists of



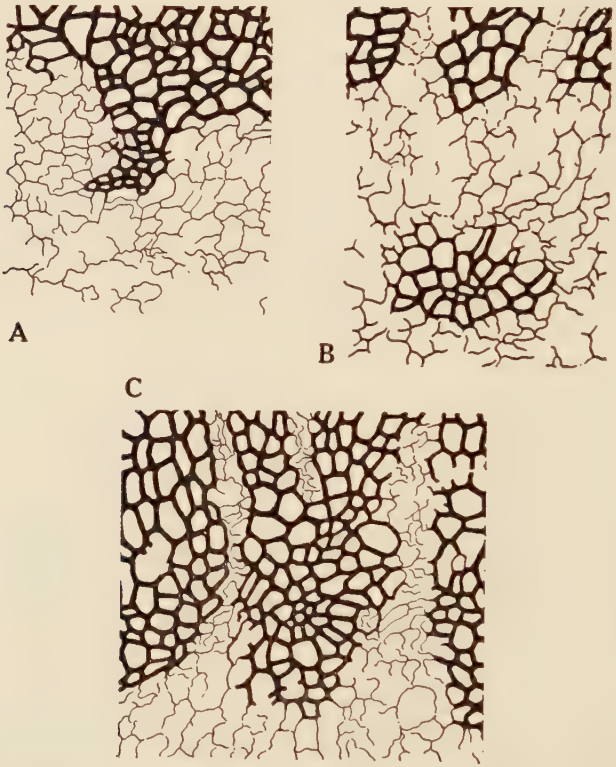
Text-fig. 1. Sclerotic nests and primary xylem of *Eristophyton waltoni*. A, B, and C, details of the sclerotic nests, all at magn. × 165, slide FSC 449. A, transverse section of cells of the outer zone showing the thick wall and small lumen. In some cells the walls appear laminated. B, oblique longitudinal section through the outer zone showing radial elongation as well as thick laminated walls and narrow lumen. C, transverse section of cells of the inner mass. The walls are thinner and the cell cavity larger. In most cases the wall thickening (shaded) has shrunk into the cell cavity. D and E, parts of a primary xylem strand cut radially to show the transition from spiral through scalariform to reticulate thickening, both at magn. × 320, slide FSC 442. D, spiral protoxylem tracheides (black) with centripetal and centrifugal scalariform metaxylem. E, scalariform and reticulate centrifugal metaxylem tracheides.

some 7 to 10 strands (Text-fig. 3, A) which diminish until they are unrecognizable when traced downwards. They consist of small spiral protoxylem tracheides and larger metaxylem tracheides showing a transition from scalariform to reticulate thickening (Text-fig. 1, D and E).

When first recognizable, about 13 mm. below departure as a leaf trace (Text-fig. 2, A), the strand is in contact with the secondary wood, only about 0·09 mm. wide, and apparently endarch. At a level about 6 mm. below the departure (Text-fig. 2, B), the strand is larger, clearly mesarch, and separated

from the secondary xylem by a few pith cells. Still higher (Text-fig. 2, c and Pl. XXXIV, Fig. 4), where it is bending outwards as a leaf trace it is still larger (about 0·15 mm. wide) and fully in contact with the secondary wood again.

After this level its course as a leaf trace is so oblique (Text-fig. 3, c, and



Text-fig. 2. Primary xylem of *Eristophyton waltoni*. All figures are at magn. × 160. A, transverse section of a primary xylem strand about 13 mm. below its departure as a leaf trace. The strand is very small, in contact with the secondary wood, and apparently endarch. Slide FSC 432. B, transverse section of the strand about 7 mm. higher. It is now larger, clearly mesarch, and separated from the secondary wood by four or five parenchyma cells. Slide FSC 435. C, transverse section of the strand just entering the secondary wood as a leaf trace. It is in contact with the secondary wood again and still mesarch. Slide FSC 438.

Pl. XXXIV, Fig. 5) that it is best followed by tangential sections of the stem. At about 1·5 mm. from the pith (Pl. XXXIV, Fig. 6) it shows rather less primary xylem but a great deal of secondary xylem which it acquired as it entered the secondary wood. At this point the leaf trace attains its maximum width of about 1·0 mm. It is separated from the secondary wood on the upper side by a triangular patch of parenchyma, an extension of the pith (see also Pl. XXXIV, Fig. 5). At 2·5 mm. from the pith (Pl. XXXIV, Fig. 7) it has become surrounded by this parenchyma. Soon after this the leaf trace ceases and is

occluded. At 3 mm. from the pith (Pl. XXXIV, Fig. 8) the primary xylem is absent and there is only a little secondary xylem, and by about 3·5 mm. from the pith no xylem at all is to be seen but only an oval patch of parenchyma embedded in the secondary wood (Pl. XXXIV, Fig. 9). Finally at about 4·0 mm. from the pith (Pl. XXXIV, Fig. 10) this also has disappeared and all that remains to mark the position of the broken trace is a disturbed area in the secondary xylem.

The order of departure of the leaf traces is shown in the serial transverse sections in Text-fig. 3, A, and their arrangement is shown in a large surface section of nearly half the xylem in Text-fig. 3, B.

The evidence, which is incomplete, is consistent with arrangement in a phyllotactic spiral of 8/21.

The *secondary wood* consists of tracheides, ray parenchyma, and a little xylem-parenchyma. Growth rings occur throughout the length of the specimen. The tracheides are typically 60 μ radially by 50 μ tangentially and are known to reach 3·5 mm. in length and have pointed ends. The radial walls show 1 to 5 vertical rows of spaced or crowded bordered pits (Pl. XXXIV, Fig. 12, and Text-figs. 4, A to D), but most commonly there are 2 or 3 alternating rows which appear to be spaced and rounded in the smaller tracheides and more crowded so that they sometimes appear quite angular in the larger tracheides. The pore opening is slit-shaped and oblique, the apertures in the pits of adjoining tracheides being crossed.

Most tangential walls are unpitted, but a few of the tracheides bordering the pith and also at the ends of growth rings show similar but frequently smaller pits (Text-figs. 4, H and I). All pitting, radial and tangential, runs for most or all the length of the tracheides.

The xylem-parenchyma consists of a few vertically elongated cells with not very thick walls. Some of them show bordered pits, and sometimes such cells link two rays (Pl. XXXIV, Fig. 13, and Text-figs. 4, F and G).

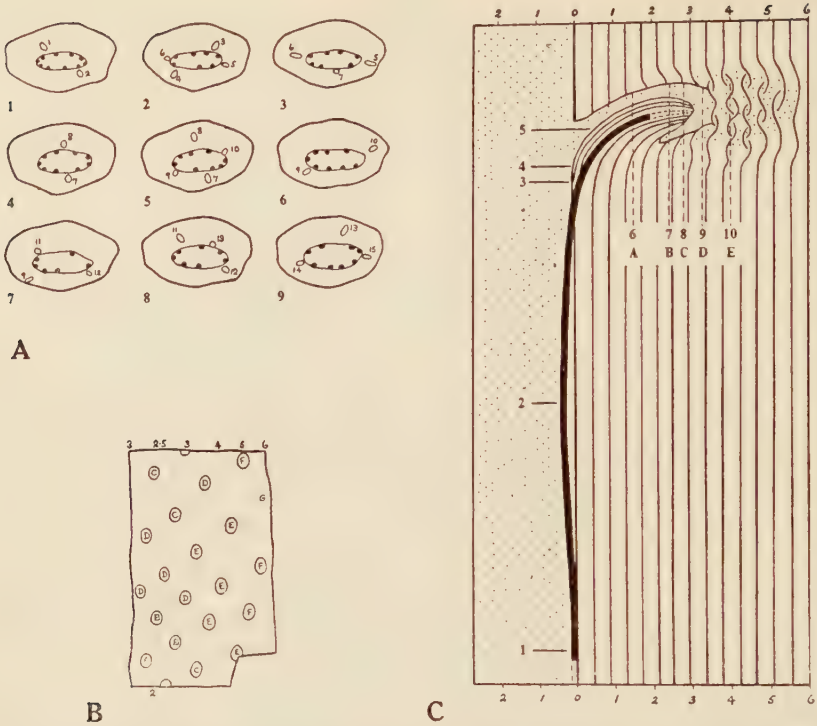
The *rays* are numerous (Pl. XXXIV, Figs. 11, 13, and 14) and the ratio of ray cells to tracheids (Gordon, 1935) is 1·0 to 2·8. They are 1 to 5 cells broad (occasionally 6 cells near the pith) and are 1 to 50 cells high. The percentage of rays of different sizes in wood away from the pith is as follows:

Width of ray:	1 cell	2 cells	3 cells	4 cells	5 cells
	31%	32%	22%	14%	1%
Height of ray:	1–4 cells	5–10 cells	11–20 cells	21–30 cells	31–50 cells
	25%	35%	27%	10%	3%

These proportions are constant except near the pith where large rays are more frequent.

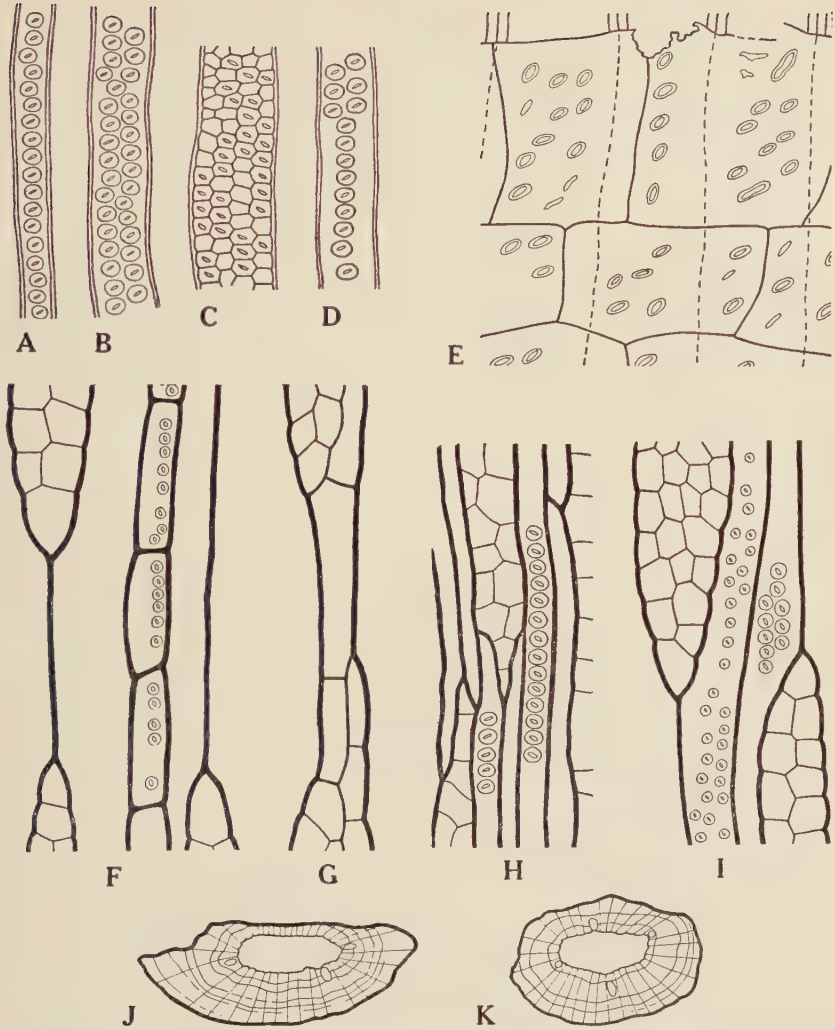
The ray field of contact between ray and tracheide shows about 6 (2 to 10) scattered oval bordered pits (Text-fig. 4, E). No pits at all were recognized on walls where one ray cell is in contact with another.

The *growth rings*, though often conspicuous, cannot always be traced right round the stem (Pl. XXXIV, Fig. 1, and Text-fig. 4, J, K). Their development appears to have been irregular, for, although similar growth rings occur



TEXT-FIG. 3. Primary xylem and leaf trace of *Eristophyton waltoni*. A, outline drawings of selected serial transverse sections from a 16-mm. length of stem showing the departure of 15 leaf traces, 2/3 natural size, slides FSC 424 to 426 and 429 to 434. Drawing 1 is from the lowest section. Sections 4 and 5 are about 1 mm. apart, the rest about 2 mm. Leaf traces are shown by rings, and primary strands (not all recognizable in every section) by black spots. B, outline drawing of a tangential section from half the surface of part of the stem showing leaf trace arrangement, 2/3 natural size, slides FSC 446 to 448. The ovals 'A' to 'D' represent leaf traces; their appearance varies with the distance from the pith (see reconstruction Text-fig. 3, C, and Pl. XXXIV, Figs. 6 to 9). The ovals marked 'E' and 'F' indicate the position of disturbed secondary wood (Pl. XXXIV, Fig. 10), and farther out, at 'G', there is no disturbance. The marginal figures indicate in mm. the approximate distances from the pith. C, diagrammatic reconstruction to scale of a primary strand and leaf trace, at magn. × 5, slides FSC 426, 428, 432, 435, 438, 439, and 441 to 448. Stippled shading = pith and parenchyma; solid black = primary xylem; broken fine lines = probable continuation of primary xylem; firm line shading = secondary wood. The inset numbers 1 to 10 refer to Text-fig. 2, A to C, and Pl. XXXVI, Figs. 4 to 10, and indicate their approximate position; the letters 'A' to 'E' refer to Text-fig. 3, B; and the marginal figures indicat edistances in mm. from the pith. Note partial separation of the primary strand from the secondary wood (exaggerated in the diagram), absence of a reparatory strand, parenchyma on upper side of the leaf trace, and distortion of the wood below and beyond the end of the leaf trace.

throughout the specimen, individual rings are not recognizable along its whole length. The best defined rings are as shown in Pl. XXXIV, Fig. 11, bottom. There is a sharp change from rather small and thick-walled tracheides to more radially elongated and thinner-walled ones. The tracheides in the dense part of one of these better defined rings ranged from 15 μ to 40 μ (mean 28 μ) radially and the first tracheides of the open part from 30 μ to 80 μ



TEXT-FIG. 4. Secondary wood of *Eristophyton waltoni*. A to D, pitting in the radial walls of the tracheides, all at magn. × 165, slide FSC 442. A, near the pith. B, C, and D, at about 3 mm. from the pith. E, pitting in the ray-tracheide fields, at magn. × 300, slide FSC 443. F to I, tangential sections, all at magn. × 165. F, a file of xylem parenchyma cells at about 5 mm. from the pith, slide FSC 448. G, xylem parenchyma connecting two rays, slide FSC 448. H, tangential pitting in tracheides at the edge of the pith, slide FSC 440. I, tangential pitting in tracheides at about 5 mm. from the pith, slide FSC 448. J and K, transverse sections showing spacing of the growth rings, both at magn. × 4/3. Leaf traces are shown by small rings but primary strands are omitted and only a few rays are indicated. J, near the base of the stem, slide FSC 423. K, near the middle of the stem, slide FSC 427.

(mean 54 μ). Similar differentiation is found in longitudinal sections, but here the growth rings are much more difficult to identify. Some of the small thick-walled tracheides show tangential pitting.

Other rings are more feebly developed and consist only of a few rows of flattish tracheides (Pl. XXXIV, Fig. 11, top).

Eristophyton waltoni sp.nov. is named after Professor Walton who collected the material.

Comparison of *Eristophyton waltoni* with Other Forms

Eristophyton waltoni is placed in the genus *Eristophyton* because it closely resembles *Eristophyton beinertianum* (Goeppert) Zalessky in all its features, in particular the parenchymatous pith with large sclerotic nests, the nature of the primary xylem system, the undivided leaf trace with single mesarch protoxylem, and the character of the secondary wood. It can, however, be distinguished from *E. beinertianum* (see Scott, 1902, 1918, 1923, and Zalessky 1911) by: (1) Details of the sclerotic nests which in the latter are not so clearly differentiated into a central core and outer zone. (2) Details of the primary xylem. In *E. beinertianum* the primary xylem strands are more numerous (17 or 18), much larger (0·15 mm. wide below to 0·85 mm. wide above), in contact with the secondary wood, and sometimes fused laterally with one another. (3) Details of the secondary wood. In *E. beinertianum* the rays are smaller, rarely more than a cell wide and not more than 26 cells high. The wood shows no well-marked growth rings such as are present in *E. waltoni*. (It is true that Scott's figures (1902, 1918) rather suggest them, but the original slides, K 677 series, merely show obscure marks due to crushing.) Finally, no evidence of xylem-parenchyma or of tangential pitting has been found in the original slides of *E. beinertianum*.

Eristophyton fasciculare (see Scott, 1902, 1918, 1923, and Zalessky, 1911) differs from *E. waltoni* in (1) its small parenchymatous pith, which has narrow elongated peripheral cells and is without sclerotic nests; (2) its larger primary xylem strands (0·22 mm. wide below to about 1·0 mm. wide above); (3) its leaf trace, whose protoxylem divides in its outward course; and (4) its secondary wood, which has only slight indications of feeble growth rings and has much smaller rays, usually 1 cell wide and up to only 16 cells high.

There are several other stems which show no medullary xylem strands or tracheides. These include:

Mesopitys tchihatcheffi (Goeppert) Zalessky; see Zalessky (1911). This Permian stem is similar to *E. waltoni* in possessing sclerotic cells in the pith, in the structure of its primary xylem and leaf trace, and its compact secondary wood with growth rings. It differs in having more scattered sclerotic cells rather than distinct nests and in its much smaller wood rays which are seldom over 1 cell wide or 9 cells high. Also the secondary xylem tracheides have only 1 or 2 rows of pits.

Endoxylon zonatum (Kidston) Scott; see Scott (1924), which is rather

similar both to *Mesopitys tchihatcheffi* and to *Eristophyton waltoni*, is described later (p. 589).

Two stems included in *Calamopitys*, namely *C. saturni* Unger and *C. radiata* Scott (see Scott, 1918, 1924, and Read, 1937), have not yet been proved to possess medullary tracheides (which characterize all the remaining members of this genus), but they differ greatly from *E. waltoni* in their larger tracheides and very large rays as well as in other features.

There are several genera which, while resembling *E. waltoni* in the pycnoxylic secondary wood, differ in possessing medullary tracheides or other indications of protostelic structure. The most similar are *Bilignea* and *Pitys*. *Bilignea* is, perhaps, deserving of special mention since one of the two species in the genus, namely *B. resinosa*, came from the same locality and horizon as *E. waltoni* (see Scott, 1924). In *E. waltoni* and *Bilignea resinosa* there is close agreement in the course and structure of the leaf traces, in the gradual change from mesarch to approximately endarch structure in the primary strands, and in the detailed structure of the secondary wood. It is practically only the pith replaced by a central column of short tracheides which distinguishes *B. resinosa* from *E. waltoni* and, indeed, this is the only feature separating the two genera. The point is dealt with fully by Scott (1924).

The genus *Pitys* (see Gordon, 1935) presents a similar case. *P. dayi*, for example, apart from its medullary primary xylem strands, is very similar indeed to *E. waltoni*. The primary xylem strands apparently do not become endarch, but apart from this, they and the leaf traces are similar, even in the manner of their occlusion, and there is agreement also in the structure of the secondary wood. In some other species of *Pitys*, e.g. *P. antiqua* and *P. withami*, the wood is even more similar. While *Pitys* and *Eristophyton* may be classified in separate families because of the important difference in their primary structures, their close agreement in other respects must raise doubts about the validity of this classification.

Discussion

1. *Morphological interest of* Eristophyton waltoni

The interest centres in the course and behaviour of the primary xylem-leaf trace strands which have been followed by the use of serial cellulose peel sections.

The first point is that there is no purely cauline primary system. The small size of the primary strands is also noteworthy and perhaps more like a modern conifer than most of the Palaeozoic stems.

Another point is that the leaf trace (the upward continuation of the primary strand) is cut off in a striking way and buried in the secondary wood a short distance from the pith. The feature is one which has been noted in a good many fossil stems, for example, *Metacordaites rigolloti* (Renault, 1896), *Archaeopitys eastmanii* (Scott and Jeffrey, 1914), *Dadoxylon arberi* (Sahni and Singh, 1926), *Callixylon zalesskyi* (Arnold, 1930), and *Pitys dayi* (Gordon,

1935)—all species which have been classified in or near to the Cordaitales. This may perhaps provide some additional support for the view, already expressed by many workers, that the genus *Eristophyton* shows an approach to the Cordaitales.

The early breakage of the leaf trace at 2–3 mm. from the pith and its burial in the secondary wood indicate that *Eristophyton waltoni* had woody stems which become bare of foliage in their lower parts. This gives some information about its habit; it is in marked contrast to such stems as *Calamopitys americana* Scott and Jeffrey (1914), for example, where the much larger divided leaf trace can be followed into the cortex and the petiolar structure is also known.

I have re-examined the sections of *E. fasciculare* and *E. beinertianum*. Both appear to me to show exactly similar abscission and occlusion of the leaf trace, though not, of course, at the same distances from the pith. Further, I have examined the wood of twigs of *Picea nigra* Link. and found that this showed essentially similar behaviour. There is thus no reason to invoke injury by volcanic fumes or ashes, as has been occasionally done with other Scottish Lower Carboniferous plants, to explain this feature.

The small size of the leaf trace of *E. waltoni* (which at its largest is little more than 1·0 mm. wide, including secondary wood, and about 0·8 sq. mm. in area) suggests a rather small leaf. Nevertheless, supposing the leaf was simple, it was probably appreciably larger than those of modern conifers as the following comparisons imply:

Species.	Leaf dimensions.	Surface area of leaf (measured).	Size of leaf trace.*
Pseudotsuga douglasii Carr.	23 mm. × 1·5 mm.	32 sq. mm.	0·0041 sq. mm.
Taxus baccata L.	22 mm. × 2 mm.	40 sq. mm.	0·0048 sq. mm.
Torreya californica Torr.	55 mm. × 4 mm.	209 sq. mm.	0·0258 sq. mm.
Cephalotaxus fortunei Hook.	70 mm. × 5 mm.	290 sq. mm.	0·0315 sq. mm.
Podocarpus milanjianus Rendle	63 mm. × 9 mm.	520 sq. mm.	0·0455 sq. mm.
Agathis australis Salisb.	70 mm. × 40 mm.	2,200 sq. mm.	0·134 sq. mm.

* The 'Size of leaf trace' has been obtained by measuring the area of xylem in a transverse section of the trace at the outer edge of the secondary wood of the stem.

The species of *Calamopitys* (see Scott, 1923; Read, 1937) have large divided leaf traces which reach a width of 4·5 mm. or more in the cortex; they presumably had a much larger leaf than *E. waltoni*. Species of *Cordaites* and *Lyginopteris* also have rather larger and divided traces and are known to have large leaves. It is thus reasonable to infer that the leaf of *E. waltoni* was larger than that of *Podocarpus milanjianus* or *Agathis australis*, but probably smaller than the leaves of such forms as *Cordaites principalis* (Germar) or *C. lingulatus* Grand'Eury (see Seward, 1917). If the correlation between size of leaf trace and leaf area suggested above holds for larger traces, then *E. waltoni* may have had a leaf as much as 250 mm. by 50 mm. (about 10 in. long by 2 in. wide). Arnold (1930) has arrived at a rather lower estimate for the leaves of *Callixylon zalesskyi*, a stem which *E. waltoni* resembles, especially in the structure and behaviour of the leaf traces.

2. *The classification of the genus* Eristophyton

Eristophyton is still no more than an isolated leafless stem. The facts resulting from the description of the new species, *E. waltoni*, seem to me to make a more compact group than before and to support Zalessky's separation of it from *Calamopitys*. Important differences are (1) the lack of medullary tracheides, (2) the reduction of the primary xylem strands to small endarch bundles when traced downwards, (3) the undivided leaf trace which was early cut off (it should not be forgotten, however, that in *E. fasciculare* the protoxylem of the leaf trace divided before the level of abscission), and (4) the compact structure of the secondary wood. In so far as such a stem genus can be classified I would group it with *Bilignea*, *Endoxylon*, and *Mesopitys* and place the whole group in the Cordaitalean complex. The possibility that it is a Pteridosperm or even a Pteridophyte cannot, however, be excluded.

Endoxylon zonatum (Kidston) Scott

Endoxylon zonatum was known from a single specimen from the Carboniferous Limestone of Dalry, Ayrshire. It was fully described by Scott (1924) and more recently by Andrews (1940). The present specimen was found some 25 miles north of the Type locality; it is a fragment, 6 cm. long by 2 cm. wide, preserved in calcareous ashy silt. Some of its bark is still present, but it has neither leaf nor branch. It is numbered Pb. 2369 (cut into two parts) in the Hunterian Museum and the sections are numbered 452 to 464 in the Figured Slide Collection, Department of Botany, University of Glasgow.

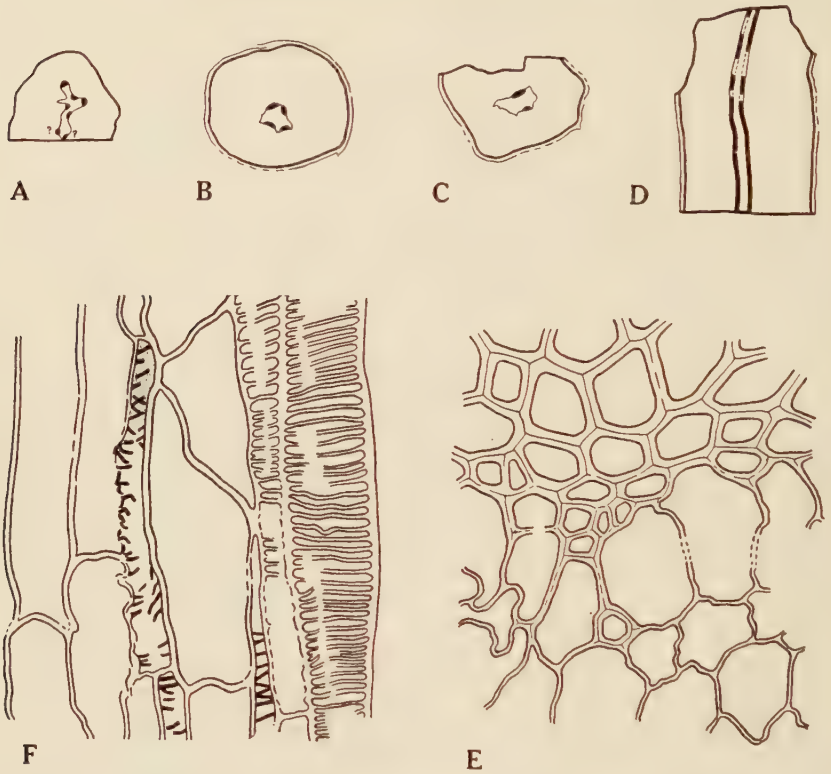
The present specimen (Pl. XXXIV, Fig. 15) closely resembles the Type and its description takes the form of a comparison with it.

The *medulla* is badly crushed but must have been only about 5 mm. wide as against 10 mm. in the Type. It is a continuous parenchyma composed of thin-walled cells 300μ long by 150μ to 200μ wide. The outer cells are narrower and thicker-walled (Pl. XXXIV, Fig. 16, and Text-fig. 5, E, F). In the Type the pith cells are often broader than high. There are no medullary tracheides or sclerotic cells at all. The *primary wood* probably forms about 7 strands, but in most sections only 2 to 5 are clearly seen owing to crushing (Text-fig. 5, A to D). They are large, endarch, widely separated, and situated in extended contact with the secondary wood. The Type had 8 similar strands.

The best preserved strand is shown in transverse section in Pl. XXXIV, Fig. 16. It measures 1·5 mm. tangentially by 0·3 mm. radially and consists mainly of uniform rather large metaxylem tracheides with smaller and thicker-walled ones along the inner margin. The protoxylem is represented by very small tracheides, some along the inner edge of the strand and others detached from the strand and situated in the outer part of the pith (Pl. XXXIV, Fig. 16, and Text-fig. 5, E, F).

In longitudinal section the primary metaxylem has well-preserved scalariform thickening (Text-fig. 5, F) as in the Type. The protoxylem has been

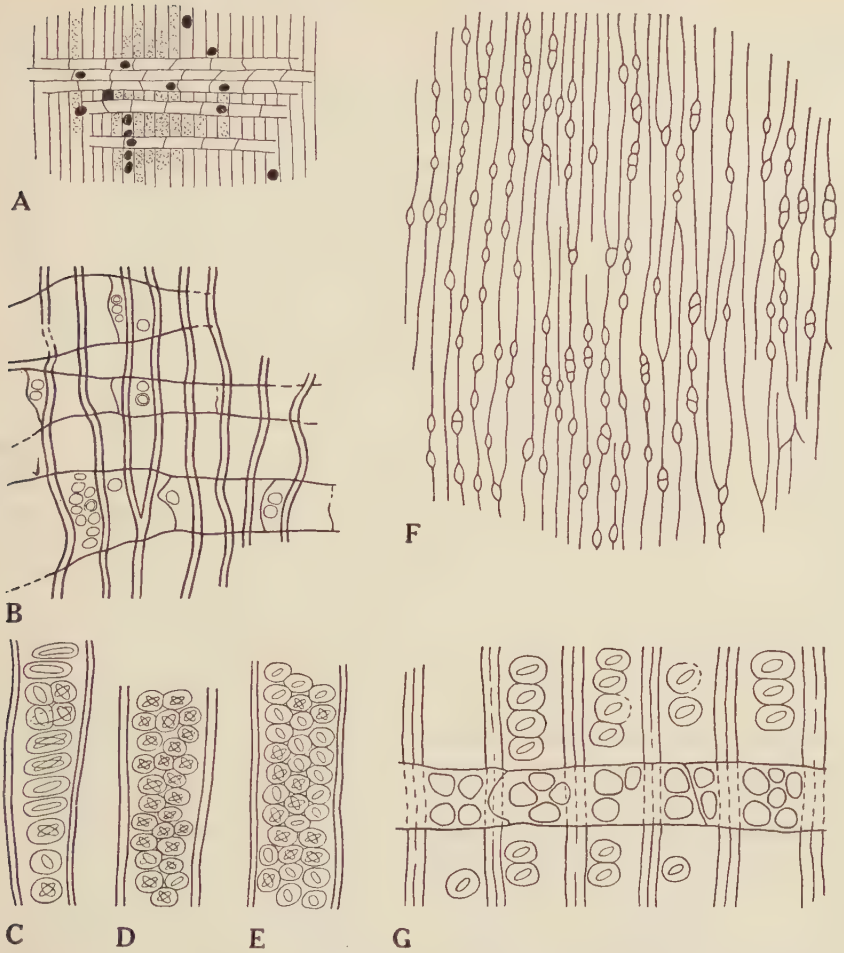
imperfectly seen, but a few pieces of spiral or annular thickening have been seen in very narrow cells both adjacent to and separated from the inner edge of the strand (Text-fig. 5, F). No evidence of mesarch strands has been found



TEXT-FIG. 5. Primary xylem of *Endoxylon zonatum*. A to D, outline drawings showing the arrangement of the primary strands (black), all natural size. A, transverse section from the upper end of Text-fig. 5, D, showing seven strands, slide FSC 455. The two strands marked '?' were proved to be primary xylem by longitudinal sections (Text-fig. 5, D). B, transverse section from near the middle of the specimen showing four strands, slide FSC 452. C, transverse section from the lower end of Text-fig. 5, D, showing only two strands, slide FSC 454. D, composite drawing from two longitudinal sections at the edge of the pith, slides FSC 459, 460. The strands shown are those marked '?' in Text-fig. 5, A. E and F, details of the primary xylem strands, both at magn. × 320. E, part of the strand in Pl. XXXIV, Fig. 16, showing small tracheides at its inner edge and also mixed with pith parenchyma, slide FSC 454. F, radial section of part of a primary strand, slide FSC 463. Note the scalariform metaxylem and poorly preserved protoxylem (thickening in solid black) both at the inner edge and separated by parenchyma.

in the new specimen and, after a re-examination of the Type, I am inclined to agree with Scott (1924) that the appearance of mesarchy in two strands of the Type (slide 803) is due to small pith cells in the centripetal position. Andrews (1940), however, believes there is some evidence of mesarchy. No leaf traces have been found in the new specimen.

The *secondary wood* is compact and looks very like that of the Type except



TEXT-FIG. 6. Secondary wood of *Endoxylon zonatum*. A, radial section showing the general appearance of the wood, at magn. × 50, slide FSC 461. Dark globular bodies of unknown nature are present in some of the cells. B, radial section showing expansion of the ray cells near the pith, at magn. × 150, slide FSC 464. Only the best preserved pits in the field are shown, and some of these have a suggestion of a narrow border. C, D, and E, tracheide pitting in radial section, all at magn. × 320, slide FSC 464. C, horizontally elongated pits in tracheides near the pith. D, irregular biseriate and triseriate pitting. E, more regular triseriate pitting. F, tangential section at about 3·5 mm. from the pith, at magn. × 70, slide FSC 458. Note the preponderance of one-celled rays and the presence of oblique walls in some of the larger rays. G, radial section showing uniseriate pitting and pitting in the ray fields, at magn. × 300, slide FSC 462.

that it shows no growth rings in some 8 mm. of wood. The tracheides are pointed and their radial walls usually show 1 or 2 rows of crowded bordered pits; occasionally there are 3 or 4 rows in a rather irregular arrangement (Text-fig. 6). In tracheides near the pith some of the pits are horizontally elongated (Text-fig. 6, C). Pits occur over the whole length of the tracheide

and no pits were seen on tangential walls. In the Type the pitting is essentially the same except that tracheides with multiseriate pits are much more common.

The *rays* are small and very numerous; the majority are but one cell broad and high (Text-fig. 6, A, F, G). Where rays 2 or 3 cells high occur the walls between the cells are often more or less oblique as seen in tangential section (Text-fig. 6, F). The rays in the Type specimen are very similar but there are slight differences which are set out below as percentages of rays of each size:

	1 cell high.	2 cells.	3 cells.	Over 3 (max. 9)	Biseriate rays.	Oblique walls.
Type specimen .	73%	17%	6%	4%	3%	50%
New specimen .	91%	6%	3%	—	—	23%

(The figures for the Type specimen are from a count of 200 rays from slide 804, but the distance from the pith is not known; and for the new specimen the count is of 400 rays from four points all at 3 to 4 mm. from the pith.)

The ray cells are thin-walled and show no pits between one ray cell and another, but the ray field of contact with a tracheid shows 2 to 10 mostly simple large round or oval pits sometimes alternately arranged (Text-fig. 6, B, G). Occasionally there is a suggestion of a narrow border round the pits of the ray field (Text-fig. 6, B). Where the rays reach the pith the cells are enlarged and give a distorted appearance to the secondary wood in this region (Text-fig. 6, B).

Tissues outside the secondary wood. Immediately outside the secondary wood is a space which presumably represents the position originally occupied by the cambium and phloem. This is succeeded, as in the Type, by a bark in which several concentric layers of periderm can be recognized (Pl. XXXIV, Fig. 15, and Text-fig. 5, B to D). Radial and tangential sections show that the cells of the periderm layers are quite short and scarcely longer than wide, but the nature of the cells between the periderm layers is not known.

The new specimen has already been compared with the Type and important points of agreement have been noted. The chief differences seem to me:

1. The absence of growth rings.
2. Moderate differences in dimensions of the pith, in the proportions of different sizes of rays, and in secondary tracheide pitting.

The difference in growth rings I think more important. The Type showed at least 14 rings in about 20 mm. of wood, 3 in the first 6 mm.; so that the 8 mm. of wood in the present specimen might have been expected to show some. It does not, however, exclude the possibility that the new specimen shows a very broad first ring only.

The differences in dimensions, proportions, and pitting are not necessarily important, for just such differences are seen in different parts of recent wood. Thus in the rather similar wood of *Araucaria araucana* Koch and in *Picea nigra* Link. I noted that the pith of a leader was twice as wide as that of a lateral of the same age and that there were differences at least as great in the tracheides and in the rays. For these reasons it has seemed to me reasonable

to identify this new specimen with the Type and accordingly to broaden its diagnosis slightly. If, alternatively, it were regarded as a second species of *Endoxylon*, the only difference would be a corresponding broadening of the generic diagnosis and as this is involved in any case I give an emended generic diagnosis below.

Emended Diagnosis of the Genus *Endoxylon* Scott

Woody axis. Medulla 5 mm. to 1 cm. wide, continuous, parenchymatous, without medullary tracheides or sclerotic cells. Primary xylem strands about 8, rather large, widely separated, lying in extended contact with secondary wood, endarch, or possibly occasionally very slightly mesarch. Leaf traces undivided, widely separated. Secondary wood compact, sometimes showing growth rings, with narrow tracheides and very small rays. Bordered pitting of the secondary tracheides in 1 to 4 crowded vertical rows on the radial walls. Rays parenchymatous, mostly uniseriate and only 1 cell high, occasionally biseriate and up to 9 cells high. Some walls between cells of larger rays oblique as seen in tangential section. Ray field with 2 to 10 large round or oval pits. Well-developed bark of successive layers of periderm.

Comparison of *Endoxylon* with Other Genera

As stated on pages 586 and 587, I recognize some agreement between *Endoxylon* and the genera *Bilignea*, *Eristophyton*, and *Mesopitys*. *Bilignea* has very similar secondary wood, particularly in the very small rays—a feature which has been emphasized by Andrews (1940), but it differs from *Endoxylon* especially in its medullary tracheides. *Eristophyton* agrees with *Endoxylon* particularly in the absence of any medullary tracheides, but important differences between the two lie in the far more numerous leaves and smaller primary strands of *Eristophyton* as well as its larger rays. *Mesopitys* is even more like *Endoxylon* than *Eristophyton*, having similar primary strands and closely similar secondary wood. The chief differences are in the sclerotic pith and smaller primary xylem strands of *Mesopitys*, differences which to my mind scarcely warrant generic separation.

The genus *Calamopitys* is readily separated from *Endoxylon* as it is now restricted to types with (or probably with) medullary tracheides and very different secondary wood (see Read, 1937).

With regard to its classification I can only repeat that I would tentatively group *Endoxylon* with *Mesopitys*, *Eristophyton*, and *Bilignea* and associate them all with the Cordaitales. This view finds a certain amount of support in the writings of various authors, particularly Zalessky (1911, p. 24), Seward (1917, p. 200), Kidston (1923, pp. 19, 20), and Andrews (1940, p. 82); but in the absence of reproductive organs I suggest this classification without any strong conviction.

It may be pointed out that there must be many genera of petrified stems with Gymnospermous secondary wood, particularly in the Lower Carboniferous; stems which range in structure from what I would call typically

Cordaitalean to typically Pteridospermic, while others approach the Pteridophytes. Any advance in understanding such stems would be welcome.

Summary

1. Two petrified stems from the Calciferous Sandstone Series, Lower Carboniferous, of the Kilpatrick Hills, Dunbartonshire, Scotland, are described from serial cellulose peel sections.

2. One of these stems, *Eristophyton waltoni* sp. nov., is close to *Eristophyton beinertianum* (Goeppert) Zalessky, but is distinguished by various details. The chief new facts relate to the behaviour of the leaf trace and its occlusion.

3. It is argued that the facts shown by the new species support the separation of *Eristophyton* from *Calamopitys*.

4. The second stem is identified as *Endoxylon zonatum* (Kidston) Scott, but differs from the Type in having no growth rings and also shows small differences in the pith and xylem. It indicates the range of variation in the genus and an emended generic diagnosis is given.

5. It is shown that the genus *Endoxylon* is readily separated from *Calamopitys*, *Bilignea*, and *Eristophyton*, but the separation from *Mesopitys* is unsatisfactory.

6. It is suggested tentatively that both *Eristophyton* and *Endoxylon*, together with *Bilignea*, all genera formerly included in the Calamopityaceae (Pteridospermales) should now be grouped with *Mesopitys* and classified in the Cordaitales.

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DESCRIPTION OF PLATE XXXIV

Illustrating W. S. Lacey's article on 'Scottish Lower Carboniferous Plants: *Eristophyton waltoni* sp. nov. and *Endoxylon zonatum* (Kidston) Scott from Dunbartonshire'.

FSC., Figured Slide Collection, Department of Botany, University of Glasgow.

All figures are from untouched photographs of peel sections, except figures 2 and 3 which are from ground rock sections.

Eristophyton waltoni sp.nov. (Figs. 1 to 14)

Fig. 1. Transverse section from the middle of the specimen showing crushed pith with sclerotic nests, secondary wood with indications of growth rings, and two leaf traces. (×3.) FSC 428.

Fig. 2. Transverse section from the upper end of the specimen. The pith, which is larger and less crushed, shows many sclerotic nests which vary greatly in shape and size. (×3.) FSC 450.

Fig. 3. Transverse section of one sclerotic nest showing the central core of ill-preserved cells and outer zone of thick-walled cells. (×24.) FSC 449. (See also Text-fig. 1, A to C.)

Fig. 4. Transverse section of a leaf trace just entering the secondary wood. (×65.) FSC 426.

Fig. 5. Transverse section of a leaf trace (the upper one in Fig. 1) at about 1·25 mm. from the pith. The trace shows a little primary xylem surrounded by secondary wood. On its inner side is a parenchymatous extension of the pith and on the outer side horizontally running tracheids can be seen. (×12·5.) FSC 428.

Fig. 6. Tangential section through the secondary wood at about 1·5 mm. from the pith and showing a leaf trace cut transversely. Note the primary xylem (*x*) of the leaf trace surrounded by secondary wood, and the parenchymatous area (*p*) on the upper side. (×24.) FSC 439.

Fig. 7. A similar section at about 2·5 mm. from the pith. The xylem of the leaf trace is now surrounded by parenchyma. (× 24.) FSC 446.

Fig. 8. A similar section at about 3·0 mm. from the pith. The secondary xylem of the leaf trace is now much reduced. (× 24.) FSC 446.

Fig. 9. A similar section at about 3·5 mm. from the pith. The leaf trace is now broken and its end covered by parenchyma. (× 24.) FSC 446.

Fig. 10. A similar section at about 4·0 mm. from the pith. Contorted tracheides and parenchyma succeed the parenchymatous area shown in Fig. 9. (× 24.) FSC 446.

Fig. 11. Transverse section of the secondary wood showing rays, tracheides, and growth

rings. A well-defined ring (*d*) is shown at the bottom of the figure and a feebly developed ring (*f*) at the top. (× 45.) FSC 428.

Fig. 12. Radial longitudinal section of the secondary wood showing multiseriate bordered pitting of the tracheides. (× 180.) FSC 443.

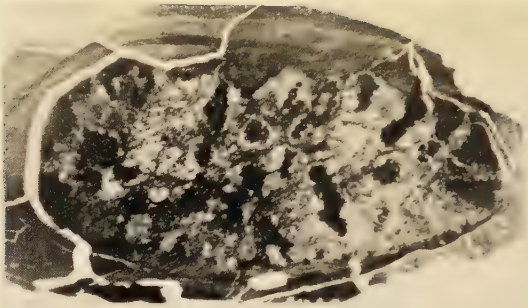
Fig. 13. Tangential section of the secondary wood at about 5 mm. from the pith showing rays cut transversely and xylem parenchyma (*xp*). (× 24.) FSC 448.

Fig. 14. Radial longitudinal section of part of a ray. (× 65.) FSC 444.

Endoxylon zonatum (Kidston) Scott (Figs. 15 and 16)

Fig. 15. Transverse section from near the middle of the specimen, showing pith, two primary strands (*x*), secondary wood, and periderm. (× 3.) FSC 452.

Fig. 16. Transverse section of one primary xylem strand, showing centrifugal metaxylem (*mx*) in extended contact with the secondary wood (*sx*). The protoxylem (*px*) occurs at the inner edge of the strand and mixed with the parenchyma of the pith (*p*). (See also Text-fig. 5, E and F.) (× 50.) FSC 454.



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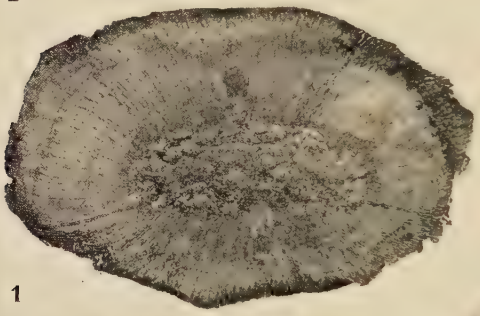

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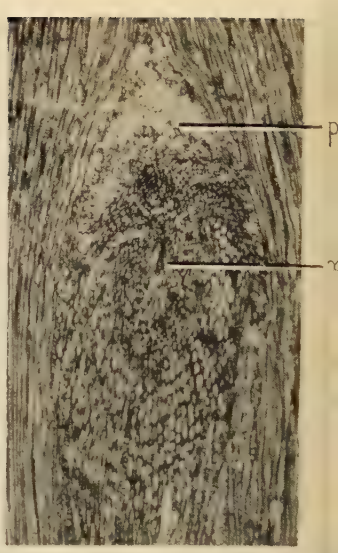

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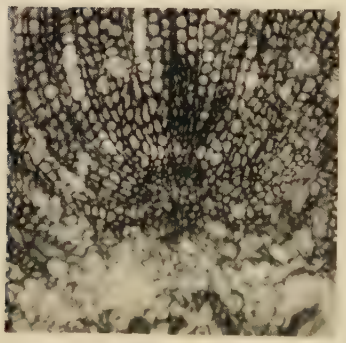

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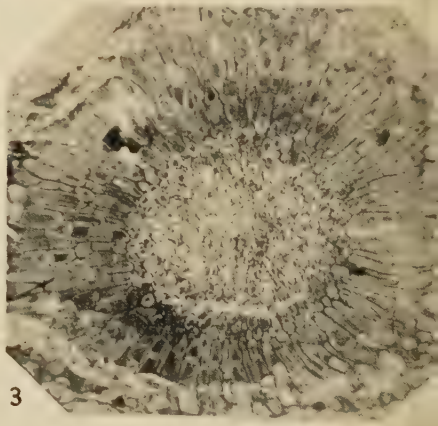

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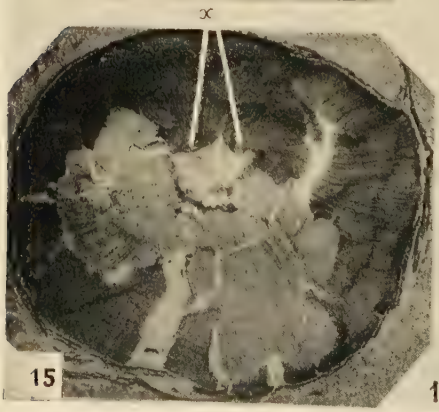

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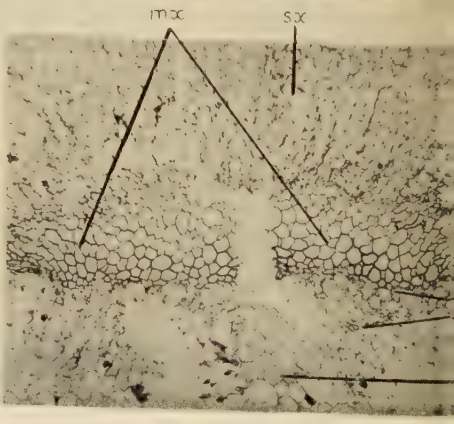

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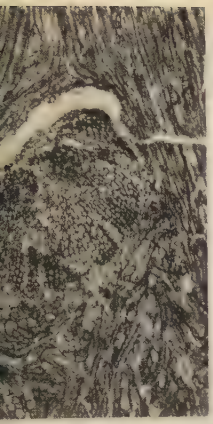

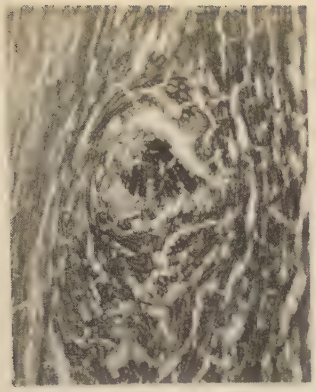

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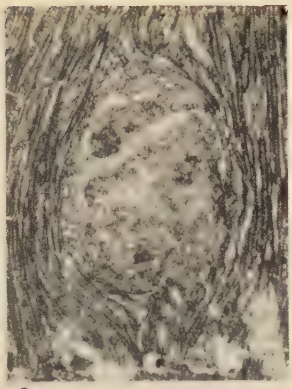

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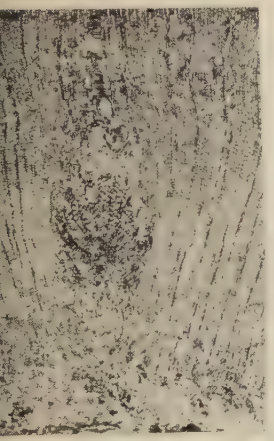

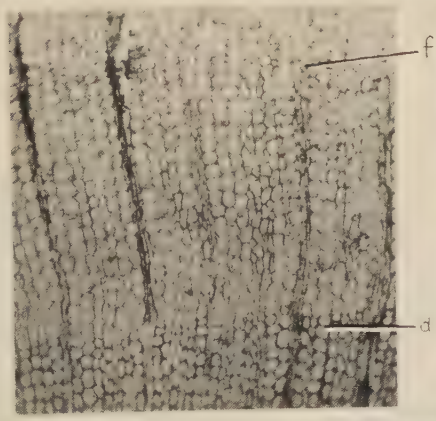

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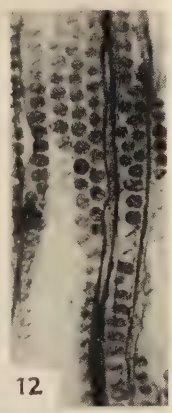

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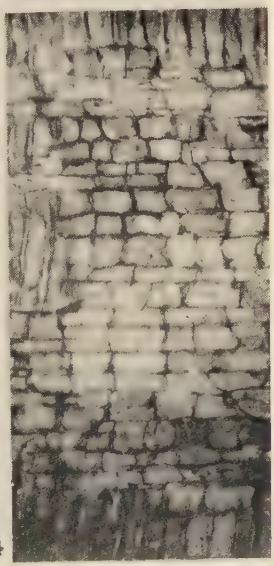

14

The Diatom Genus *Tabellaria*

III. Problems of infra-specific Taxonomy and Evolution in *T. flocculosa*

BY

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With four Figures in the Text

ABSTRACT

All degrees of morphological and ecological differentiation exist between attached and planktonic populations of *T. flocculosa* in various lakes. *T. flocculosa* var. *asterionelloides* is regarded as a group of euplanktonic strains in which the star-shaped colony form has a genetic basis. The relationships of different strains are discussed in relation to (*a*) Wesenberg-Lund's views on the littoral origin of star-shaped colonies, and (*b*) the evolution of euplanktonic strains.

INTRODUCTION

ACCORDING to Wesenberg-Lund (1908), star-shaped colonies of *Tabellaria* (i.e. *T. flocculosa* var. *asterionelloides* (Grun. in V. H.) Knud.) are ephemeral in the plankton and are derived annually from zigzag attached ones (i.e. *T. flocculosa* (Roth) Kütz. var. *flocculosa*). This hypothesis has led to the widespread view (e.g. Skuja, 1948) that the star-shaped colonies are merely an environmental modification of the 'main species' and should, therefore, be given the rank of forma. Nevertheless, W. and G. S. West (1909) concluded from their studies on Windermere that the star-shaped colonies are perennial in the plankton of this lake and are unrelated to the attached zigzag colonies in the littoral regions.

The purpose of this paper is (*a*) to describe an investigation of this problem in the English Lake District and elsewhere; (*b*) to relate the results to the taxonomy of infra-specific groups in *T. flocculosa*; and (*c*) to discuss their evolution.

Use of terms. The existence of morphologically distinct strains of *T.*

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flocculosa has been described earlier (Knudson, 1952, p. 431). The term 'strain' is used in these papers for a group of individuals which, on account of their morphological similarity, are believed to be genetically homogeneous. Differences between frustules due merely to valve-length are not used to separate strains nor are differences which can be traced to the direct influence of the environment.

Indirect evidence that some of the morphological differences between strains are genetic ones has been obtained by showing that (*a*) morphologically distinct strains coexist in the same natural environment (e.g. the plankton of a particular lake) for long periods of time, each strain having characteristic valve-length properties (Knudson, 1953, p. 139), and (*b*) under identical conditions clone cultures made from these strains continue to differ in certain morphological features (e.g. apical/pervalvar axis ratio, ibid., p. 149). A *clone culture* is one started from a single *Tabellaria* colony.

Characters subject to environmental modification have been found in two ways: (*a*) by observing the variation in natural samples throughout the year, and (*b*) by varying the physical and chemical conditions of clone cultures grown in the laboratory.

The term *population* is used for the individuals of a single strain in one particular locality, e.g. the plankton of a lake. A population may consist of one or more length groups (cf. Fig. 1). Distinct length groups can only be recognized when (*a*) the periods between auxospore formation are sufficiently long, and (*b*) the post-auxospore cells are of fairly uniform valve length. A length group is composed of one or many *clones*, each clone being derived from an auxospore. Whereas a population is limited to one particular locality, a strain may be found in many bodies of water, which are not necessarily connected.

The Ecological Relationships of Populations of *T. flocculosa*

Investigations have been made of (*a*) the morphology of attached and plankton samples of *T. flocculosa* in order to find which strains, if any, are confined to the plankton, and (*b*) seasonal variations in the numbers of cells per ml. of the different strains in the plankton. Samples from the main lakes in the English Lake District and one in Scotland (Loch Ness) have been examined at intervals not exceeding 4 weeks for at least a year. No two lakes with identical *Tabellaria* floras have been found and only four are described in detail. These four have been selected because they form a series in which there is an increasing degree of differentiation between planktonic and attached material.

Methods

Plankton samples for quantitative purposes were collected with a plankton net and concentrated on a Whatman 541 filter-paper; in Windermere and Blelham Tarn samples were collected by boat, elsewhere from a steeply

shelving shore. Plankton samples for quantitative purposes were collected with a 5-m. rubber tube (Lund, 1949, p. 390) in Windermere and Blelham Tarn, elsewhere by means of a throw-out bottle. Numbers of cells of the different strains were estimated in the manner described by Lund (1949, p. 391).

Samples of attached *Tabellaria* were collected from *Phragmites communis*, *Schoenoplectus lacustris*, or *Equisetum fluviatile*. The vertical shoots of these plants were cut about 35 cm. below the water surface and the lower portions of the cut shoots put into a bottle. The small amount of lake water inevitably introduced by this procedure was drained off before the sample was examined. Despite this precaution some contamination by planktonic algae occurs because they become entangled in the epiphytes. In the very rare instances when it was uncertain whether a strain was truly planktonic, the ratio of colonies of this *Tabellaria* to some undoubtedly planktonic alga was found, both in the plankton and epiphytic samples. *Asterionella formosa* was particularly suitable for this purpose owing to its similar form. On the basis of whether these ratios were significantly different the following definition of a euplanktonic strain is found useful: a euplanktonic strain is one which is overwhelmingly planktonic; its presence in epiphytic samples is only accidental due to its being entangled there together with other euplanktonic organisms in the same proportions as in the open water.

Results

(*a*) *Loughrigg Tarn.* Epiphytic and plankton samples are composed entirely of zigzag colonies of untwisted cells. Length-frequency histograms based on 100 measurements of valve length (from 100 colonies selected at random) are shown in Fig. 1. The mean and standard deviations of the two samples are: epiphytic sample, $36{\cdot}1\,\mu \pm 4{\cdot}4\,\mu$; plankton sample $35{\cdot}8\,\mu \pm 5{\cdot}7\,\mu$. There is no significant difference between these two means.

Although cells from the two samples are apparently identical in all respects, daughter cells in the epiphytic sample seem to separate more slowly than those in the plankton sample. Thus 36 per cent. of the cells in the epiphytic sample were still connected by their valve faces, whereas only 15 per cent. of the cells from the plankton were. Although this difference is sufficiently well marked to be detectable without counting, it is very unlikely to have a genetic basis. Thus Vollenweider (1950, p. 229) has shown that the number of cells in a zigzag colony and the proportion of cells forming bands (i.e. blocks of unseparated cells) increases with the calcium concentration of the culture fluid. We may conclude then that there is a single strain in Loughrigg Tarn, and that there is no positive evidence that there are distinct epiphytic and planktonic populations.

(*b*) *Bassenthwaite Lake.* Epiphytic and plankton samples consist of zigzag colonies of untwisted cells, but star-shaped colonies are formed sporadically in the plankton (Fig. 2, *A*). The length-frequency histograms of the two samples are strikingly different (Fig. 1), but the cells are apparently identical.

A single strain seems to be present, but there are two possible explanations for the differences in the length-frequency histograms. Either there are two length groups of which the longer is more abundant in the plankton and the shorter on the reeds, or else there are two physiologically distinct populations,

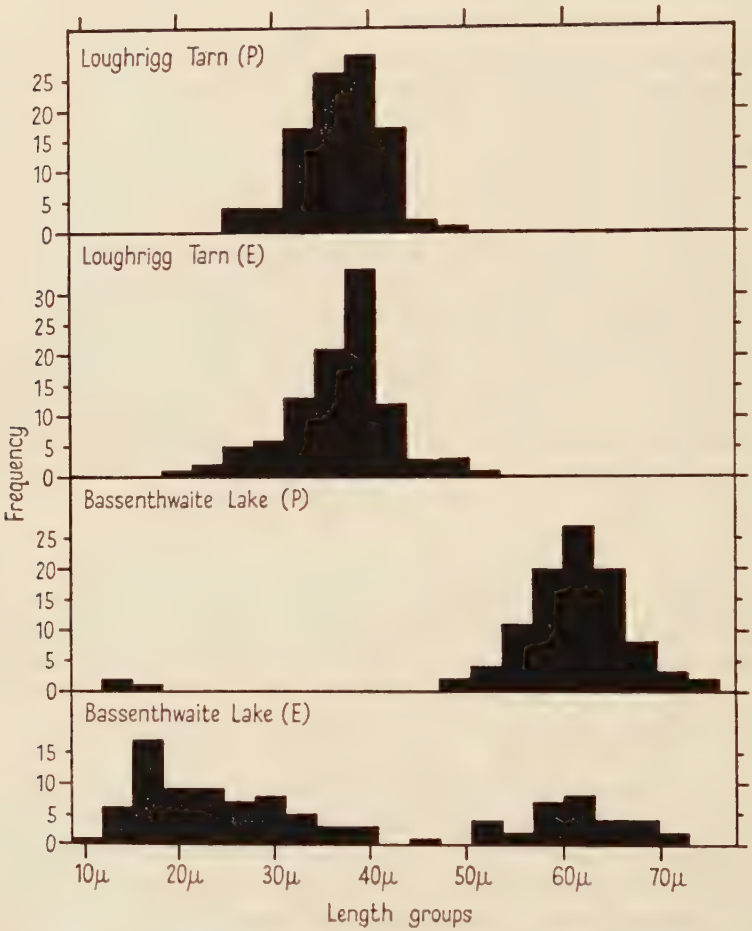

FIG. 1. Length-frequency histograms based on 100 measurements of valve-length in plankton (P) and epiphytic (E) samples of *T. flocculosa* from Loughrigg Tarn and Bassenthwaite Lake.

one attached, the other planktonic, which reproduce independently. The first hypothesis suggests that the shorter cells do not flourish in the plankton: nevertheless, cells of lengths which are absent in the plankton of Bassenthwaite Lake are present in considerable numbers in the plankton of Loughrigg Tarn (Fig. 1). The results in Bassenthwaite Lake are therefore best explained by supposing that there is a single strain and two independently reproducing populations which are physiologically, but not morphologically, distinct.

(*c*) *Loch Ness.* Epiphytic samples are entirely composed of zigzag colonies (Fig. 2 *C*), whereas plankton samples consist of star-shaped colonies showing all transitions to a zigzag colony form (Fig. 2 *B*). Samples from the two habitats also differ in (*a*) length-frequency histograms, (*b*) mean angle of

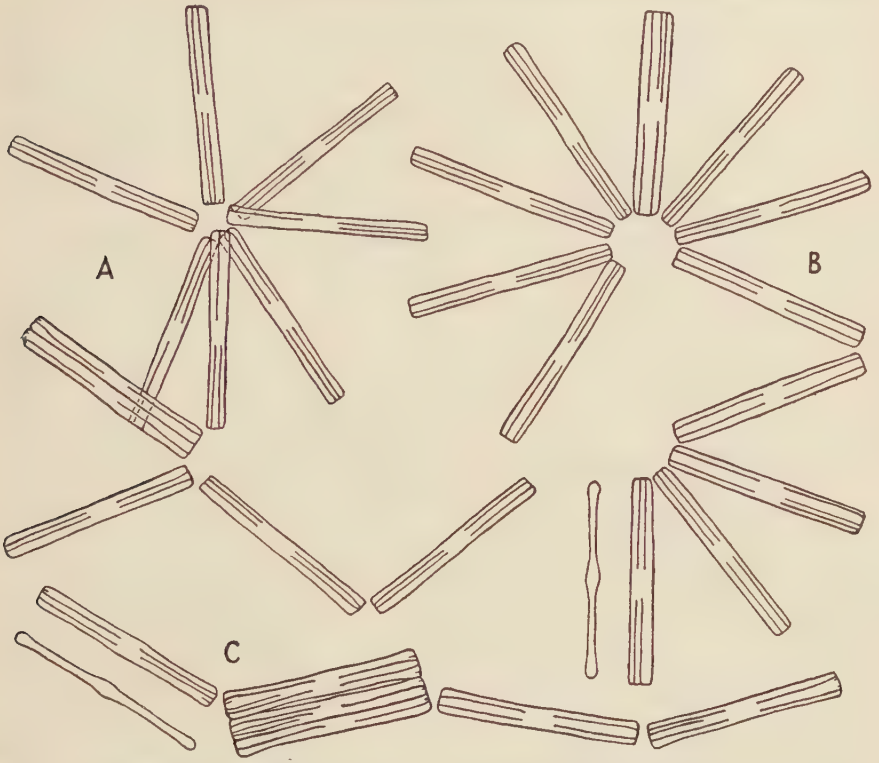

FIG. 2. *T. flocculosa*. *A*, planktonic colony (Bassenthwaite Lake); *B*, planktonic colony (Loch Ness); *C*, epiphytic colony (Loch Ness). (×410.)

divergence between the cells, (*c*) percentage of cells remaining attached by their valve faces, (*d*) mode of insertion of septa, and (*e*) degree of development of the rudimentary septa.

Whereas in Bassenthwaite Lake the star-shaped colonies occur sporadically, consist of an indefinite number of cells, and are arranged in loose helices, those in Loch Ness are present throughout the year, consist mainly of eight cells, and are of more definite structure. At least two of these characters, viz. mode of insertion of septa and ability to form 'stable' star-shaped colonies, are believed to have a genetic basis, and consequently Loch Ness has two strains of *T. flocculosa*: a euplanktonic strain (1) and a predominantly attached strain (2).

(*d*) *Blelham Tarn.* Two very dissimilar strains can be detected in the plankton (Fig. 3, *A*, *A′*, *A″* and *B*, *B′*, *B″*). They differ in at least four characters which have a genetic basis, viz. (*a*) presence or absence of 'stable'

star-shaped colonies, (*b*) degree of development of rudimentary septa, (*c*) presence or absence of frustule twist, (*d*) valve contour. In addition these two strains have different length properties. If no other samples had been examined they would probably have been described as separate species.

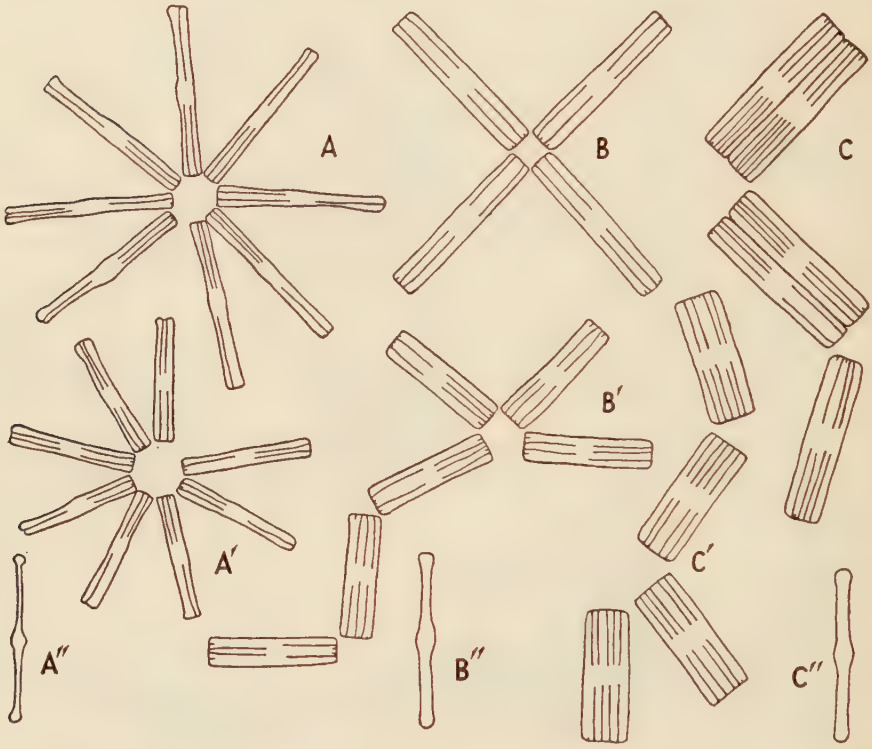

FIG. 3. Blelham Tarn: three strains of *T. flocculosa* as seen in girdle and valve view. The lettering corresponds to the strains *A*, *B*, and *C* described on p. 601. (×410.)

The epiphytic sample consists entirely of zigzag colonies of untwisted cells (Fig. 3, *C*, *C′*, *C″*). About 95 per cent. of the sample consists of a strain which differs from the plankton strains in (*a*) mode of insertion of septa, (*b*) mean number of septa, and (*c*) extent to which daughter cells remain attached by their valve faces. Since the first two of these characters are genetically controlled, it appears that there are at least three strains in Blelham Tarn: a predominantly star-shaped euplanktonic strain with twisted frustules (*A*): a predominantly zigzag euplanktonic strain with untwisted frustules (*B*), and a wholly zigzag predominantly attached strain with untwisted frustules (*C*). Strain (*C*) and the other rare epiphytic strains are only occasionally seen in the plankton.

The two euplanktonic strains differ greatly not only in form but also in growth physiology. Fig. 4 shows the seasonal variations in the number of cells per ml. of these two strains.

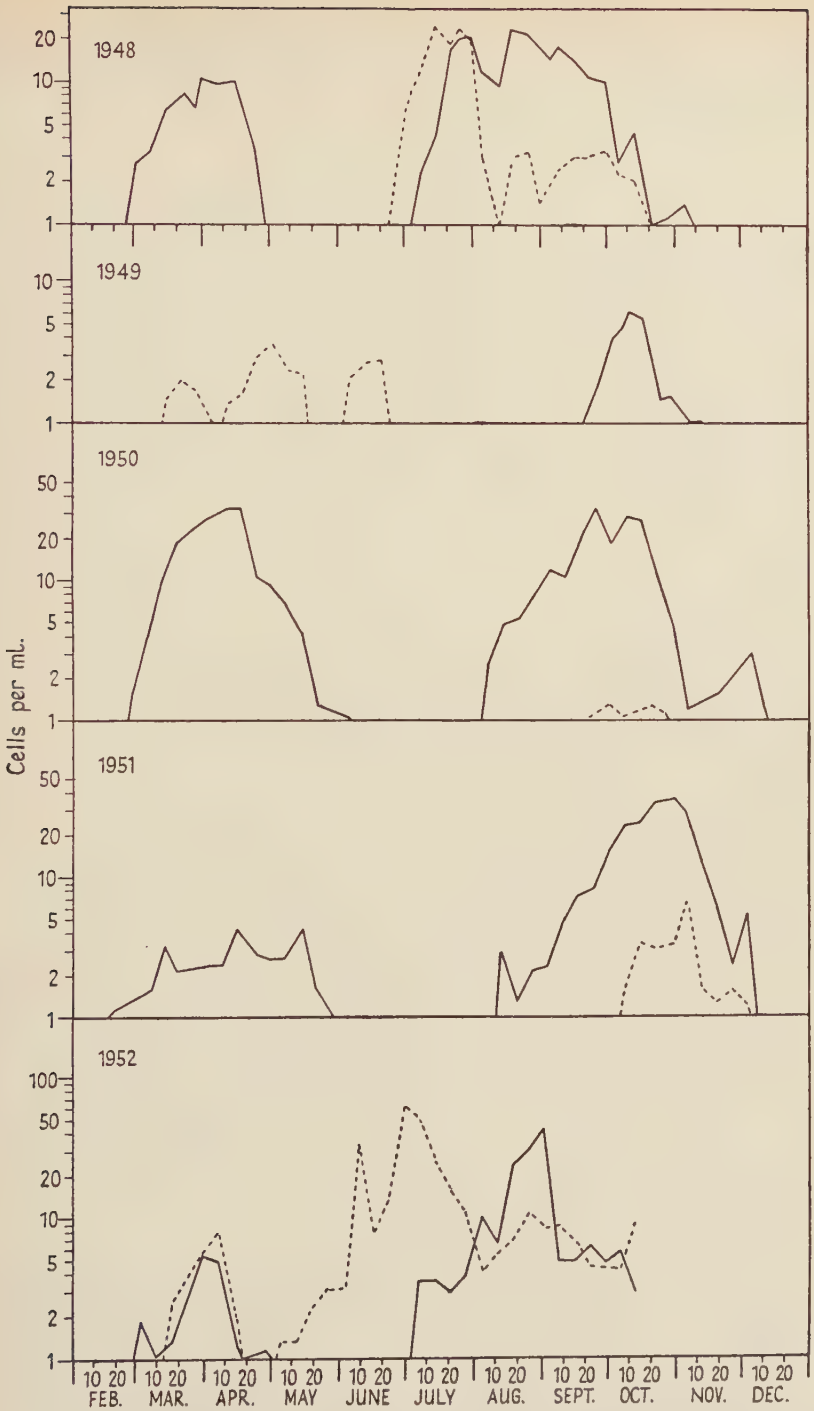

FIG. 4. Seasonal variations (1948–52) in the number of cells per ml. of two strains of *T. flocculosa* in the plankton of Blelham Tarn (plotted on a logarithmic scale). Strain *A* (var. *asterionelloides*) shown with dotted line; strain *B* (var. *flocculosa*) shown with continuous line.

(*e*) *Other lakes.* The morphology of strains of *T. flocculosa* in some other lakes has been described earlier (Knudson, 1953, p. 142).

Taxonomic Conclusions

From the descriptions and results given in the last section it is possible to classify these strains of *T. flocculosa* into two groups:

Group I. Those in which star-shaped colonies are present throughout the year, though not necessarily in larger numbers than zigzag colonies of the same strain. There is usually a definite number of cells to such star-shaped colonies (e.g. 80 per cent. of the population may consist of 8-celled star-shaped colonies), and in some there is a polarity in the development of the septa (Knudson, 1953, p. 132). These strains are well defined and can be identified even in the zigzag condition (loc. cit., p. 139). All of them are euplanktonic and the star-shaped habit is believed to have a genetic basis.

Examples: Loch Ness (1), Blelham Tarn (*A*), Windermere, Ullswater (1) and (2), Loweswater.

Group II. Those in which star-shaped colonies are formed sporadically or not at all. The number of cells to a star-shaped colony is indefinite and large maxima of star-shaped colonies (e.g. 100+ cells per ml.) are never due to strains of this group. They may be attached or planktonic in habit and the star-shaped form is believed not to have a genetic basis.

Examples: Loughrigg Tarn, Bassenthwaite Lake, Thirlmere, Coniston Water, Blelham Tarn (*B* and *C*), Loch Ness (2) and Ullswater (3).

It is now pertinent to know whether or not these groups correspond to two taxa bearing the names *T. flocculosa* vars. *asterionelloides* and *flocculosa*. The specimens to which these names are permanently attached have been described in detail elsewhere (Knudson, 1952, pp. 425–7). The neotype of *T. flocculosa* var. *asterionelloides* closely resembles the Loch Ness plankton strain, and since all known strains of this general type belong to Group I, we may conclude with reasonable certainty that Grunow's original material had (*a*) a euplanktonic habit, and (*b*) star-shaped colonies with a genetic basis and therefore ones which were well defined and present throughout the year. The neotype of *T. flocculosa* var. *flocculosa* is, like Roth's original material, an epiphytic sample of zigzag colonies. There is, then, no legitimate objection to a classification such as the following:

Euplanktonic: star-shaped colonies present throughout the year, often showing transitions to zigzag form. Star-shaped colonies with a genetic basis . . . . . *T. flocculosa* var. *asterionelloides*

Planktonic or attached: colonies predominantly zigzag; star-shaped colonies absent or sporadically produced. Star-shaped colonies ephemeral and without a genetic basis . *T. flocculosa* var. *flocculosa*

By means of this classification the vast majority of strains can readily be

named, but the advantage of doing so is still open to question. There is no doubt that *T. flocculosa* var. *asterionelloides* (as defined above) comprises strains which are morphologically and ecologically more specialized than those of *T. flocculosa* var. *flocculosa*, but the significance of the 'stable' star-shaped colonies of the former is highly problematic.

The traditional view that a star-shaped colony form is an adaptation to planktonic life must be treated with caution. If it were by itself a great asset we might expect that predominantly star-shaped strains would always compete successfully with predominantly zigzag ones, but this is not so in Blelham Tarn (Fig. 4). We might also expect that *Tabellaria* maxima in lakes where *T. flocculosa* var. *asterionelloides* was present to be considerably greater than in those having only *T. flocculosa* var. *flocculosa*. In the Lake District there is not a considerable difference between them. Thus the maximum recorded population density of *T. flocculosa* var. *asterionelloides* is 1,690 cells per ml. (Esthwaite Water), whereas that of *T. flocculosa* var. *flocculosa* is 690 cells per ml. (Bassenthwaite Lake).

The fact that euplanktonic strains are not necessarily star-shaped has already been mentioned (Blelham Tarn strain *B*, p. 602), and the extreme diversity of strains of *T. flocculosa* var. *asterionelloides* is another indication that this is not a natural group. Moreover, *T. flocculosa* var. *asterionelloides* is found in lakes of the most varied chemical and physical nature. It occurs in Loch Ness, an extremely oligotrophic lake, but also in very eutrophic water, such as the Rhine and Zürichsee. In lakes of intermediate status it may be present (Windermere) or absent (Lac Léman). The simplest explanation of these facts is that *T. flocculosa* var. *asterionelloides* is an assemblage of strains which have independently acquired a star-shaped colony form. The classification given above is probably worth retaining, but it should not blind us to the fact that *T. flocculosa* var. *asterionelloides* embraces strains which are more highly differentiated than many diatom species.

The systematic position of *T. flocculosa* var. *pelagica* (sensu Holmboe and Huitfeldt-Kaas) cannot be settled until the type material has been found, but it seems very likely that it was similar to the strains described from Thirlmere and Coniston Water. If this is so, *T. flocculosa* var. *pelagica* would be synonymous with *T. flocculosa* var. *flocculosa*.

The Evolution of Euplanktonic Strains

T. flocculosa occurs in many different types of freshwater habitat, but only a small proportion of investigated lakes has a euplanktonic strain; it is very likely, then, that the attached habit is the primitive one in this species. The number of euplanktonic strains is nevertheless large, and it is reasonable to suppose that *T. flocculosa* var. *asterionelloides* is a group of morphologically and genetically distinct strains which have evolved independently.

Loughrigg Tarn, Bassenthwaite Lake, Loch Ness, and Blelham Tarn form a series in which there is an increasing degree of speciation between planktonic and non-planktonic populations. This series can be used in support of

the hypothesis that the euplanktonic strains have evolved by a number of steps from the attached strains. If these two hypotheses are acceptable, it is worth while to speculate on the mechanism by which this could have taken place. In any body of water large enough to be significantly affected by wave action, colonies of *T. flocculosa* become detached from the shore and carried out into the open water. Whether or not these colonies flourish in the plankton must depend on such factors as their sinking rate and their ability to multiply under the different light and chemical conditions of the open water. It is inevitable that the nutrient status of the plankton colonies diverges from that of the attached ones, and this difference is likely to affect the onset of auxospore formation. As a result, two independently reproducing groups are formed; the two populations become 'out of phase' and their length-frequency histograms differ. At this stage of differentiation, which is apparently that reached in Bassenthwaite Lake, star-shaped colonies are sporadically produced and have no genetic basis.

Once two independently reproducing groups have been formed, we can envisage the planktonic strain becoming morphologically distinguishable from the attached strain by the natural selection of mutants with, say, thinner shell, star-shaped colony morphology or higher apical/pervalvar axis ratio. In Blelham Tarn the euplanktonic strain (*B*) seems to have diverged but little from the attached strain (*C*): both are predominantly zigzag and the differences in frustule morphology between them are very slight.

In Loch Ness only two genetically determined morphological differences can be detected between the planktonic and attached strains, but since the planktonic strain is predominantly star-shaped and the attached strain predominantly zigzag, samples of the two are superficially very dissimilar. At least four genetically controlled morphological differences exist between the euplanktonic strain (*A*) and the attached strain (*C*) in Blelham Tarn, and consequently they are very easily separated. In Windermere several different star-shaped strains have successively dominated the plankton in the past forty years (Knudson, 1953, p. 146), and it therefore seems reasonable to suppose that mutation and selection are processes which are continually taking place in certain lakes.

From the widespread distribution of other euplanktonic diatoms, e.g. *Asterionella formosa*, it would be very surprising if some strains were not found in more than one lake. In the English Lake District, where more than 100 bodies of water have been investigated, the most widely distributed plankton strain is the Blelham Tarn one (*A*) which also occurs in Windermere, Esthwaite Water, Bigland Tarn, and Kendal Canal, all within an area of 400 sq. km. Since these waters are of similar chemical composition, it is difficult to know whether this is an example of dispersal or convergent evolution. The strain is so distinctive morphologically, however, that the former explanation is more probably correct.

The distribution of *T. flocculosa* var. *asterionelloides* should, therefore, be related to (*a*) evolution *in situ*, and (*b*) dispersal. It is not to be assumed,

however, that a strain which evolves in one lake will necessarily flourish as well in another where different physical and chemical conditions obtain. It is noteworthy that Blelham Tarn strain (*A*) forms much smaller maxima in this lake than in Windermere. During 5 years' weekly observations the record maxima are: Windermere 630 cells per ml., Blelham Tarn 65 cells per ml. It thus seems that Windermere is a much more suitable environment for the growth of this strain than is Blelham Tarn. The absence of this strain from other lakes in the vicinity could therefore be due not only to lack of dispersal but because physical or chemical conditions are even more adverse for its establishment and growth than they are in Blelham Tarn.

T. flocculosa vars. *teilingii* and *geniculata* seem to be extreme examples of incipient species. Both are very distinct morphologically and have restricted distributions in Scandinavia. The Blelham Tarn strain (*A*) seems to be at a similar stage of differentiation to these two varieties, but since there is no proof that it is genetically stable it seems inadvisable as yet to regard it as a separate variety.

Finally it should be emphasized that the above classification of *T. flocculosa* must be regarded as a temporary measure only. In a species showing evidence of present-day evolution a perfect taxonomic system is not to be expected, but there is still remarkably little information available about the distribution of euplanktonic strains in *T. flocculosa* and none about the genetics of auxospore formation in the species. Our ignorance of these matters is so great that any intensive study of lakes with euplanktonic strains is likely to provide results of very great importance for a more rational classification of infraspecific groups in *T. flocculosa*.

Discussion of Wesenberg-Lund's Hypothesis

Wesenberg-Lund's views on the littoral origin of the star-shaped colonies may be summarized as follows from his 1908 paper: 'Littoral zigzag colonies become detached by the spring storms and pass into the plankton where they become transformed into star-shaped colonies; the star-shaped colonies perish in the plankton and are not responsible for the spring maximum of the following year.' The main facts on which this hypothesis was based were: (1) in spring zigzag colonies occurred on plants and stones in the littoral regions; (2) zigzag colonies preceded star-shaped colonies in the spring plankton; (3) star-shaped colonies predominated at the end of the growing season; (4) in the following year the organism reappeared in the zigzag form.

Investigations in the Lake District and elsewhere have failed to confirm Wesenberg-Lund's hypothesis, but his facts are readily explicable on the supposition that there were two strains in Furesø as in Loch Ness.

It is obvious from Wesenberg-Lund's excellent photomicrographs (1904, pls. 1 and 2) taken at different times of year that the zigzag colonies he observed in the early spring plankton belonged to the same morphological strain as the later star-shaped ones. The great rarity of star-shaped colonies in the winter

plankton was, perhaps, the chief reason why he suggested that the organism was recruited each year from the littoral regions. Nevertheless, the large spring maxima of *Asterionella formosa* have been shown (Lund, 1949) to arise from perennial colonies in the plankton, often very few in numbers, and this is also true of the euplanktonic strain (A) of *T. flocculosa* in Blelham Tarn.

In Fig. 4 the population densities of the two strains are shown only when the cell numbers exceed 100 per 100 ml., this being the level below which significant changes from one week to the next are difficult to detect. Nevertheless, the occasions were very few when *T. flocculosa* var. *asterionelloides* was unrecorded either in 100 ml. of sedimented lake water or in two drops of concentrated net sample. October 1949 to July 1950 was the longest period during which the records of this alga are erratic. This was also true of *Asterionella formosa*, and it is certain that this happened because insufficient material was examined rather than because the alga disappeared from the plankton.

Wesenberg-Lund's photomicrographs do not show any colonies which can be identified certainly as littoral in origin. It was thus a coincidence that the plankton strain was in a zigzag condition at the same time as the attached strain was abundant in the littoral regions. Whatever the genetic relationship of the two, it is clear that Wesenberg-Lund had suspicions about his own hypothesis, for he found discrepancies between the length-frequency histograms of the two samples. Thus he states (1908, p. 30): 'I had very much hoped that statistical investigation of the variations (i.e. in valve length) would have . . . perhaps proved the existence of a relation between the littoral and plankton form, yet I have not been so fortunate.'

Observations on clone cultures have shown that the transition from a star-shaped to a zigzag colony form can proceed in either direction in strains like that in Furesø: the transformation of star-shaped into zigzag colonies between the autumn of one year and the spring of the next was probably missed owing to the scarcity of material. The 'seasonal dimorphism' observed in Furesø is not a universal phenomenon, but in this lake it lent support to a very attractive hypothesis.

Summary

1. Some strains of *T. flocculosa* are confined to the plankton (euplanktonic, but there are all degrees of differentiation between planktonic and attached populations.

2. *T. flocculosa* var. *asterionelloides* is probably not a natural group, but the name is usefully retained for strains characterized by (*a*) the euplanktonic habit, and (*b*) star-shaped colonies with a genetic basis.

3. *T. flocculosa* is believed to show incipient speciation, the most evolved 'species in the making' being some of the euplanktonic strains.

4. No verification has been found for Wesenberg-Lund's hypothesis on the littoral origin of *T. flocculosa* var. *asterionelloides* and an alternative explanation of his facts is put forward.

Acknowledgements

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Note on the Relationship between Climatic Factors and Transpiration and Assimilation of *Eugenia aromatica*

BY

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With three Figures in the Text

AS part of an investigation into the effect of the Sudden-death disease (Nutman and Sheffield, 1949) on the physiology of the clove-tree, a detailed study of the tree's water-relations was started. Circumstances then made it necessary to concentrate on more obviously relevant lines of investigation and the physiological study was never completed. Since, however, there are few records of the relations between climatic factors and the transpiration and assimilation rates of tropical plants, these incomplete results are presented.

Transpiration was studied by the method developed by Nutman (1940). A special mechanism attached to an Avery $2\frac{1}{2}$-ton weighbridge enabled changes in weight of less than 1 in 350,000 to be recorded. The tree used was a flourishing 8-year-old sapling, estimated to carry about 20,000 leaves with a total area of about 40 square metres. It was excavated by the method described by Schmidt and Nutman (1940) and, with its root system in a monolith of undisturbed soil, was left in position on the weighbridge for several months before measurements were made. The tree showed no signs of flagging and it remained perfectly healthy for more than a year after excavation.

Climatic data were recorded as follows:

Solar radiation was measured by a photocell under an opal glass dome. The only cell available, unfortunately, showed a steady drop in sensitivity, and hence it is not possible to compare the records made on separate days with each other. Records on any one day, however, are reasonably accurate. Readings were taken every 60 seconds and recorded as 10-minute averages in arbitary units.

Wet-bulb depression was measured by a screened wet and dry thermocouple in a current of air produced by a small electric fan. Readings were taken at 60-second intervals as before and recorded as 10-minute averages.

Temperature and humidity were measured by a recording thermohygrograph in a Stevenson screen.

Figures 1 and 2 are typical of the records obtained. These are in some respects similar to those published for *Coffea arabica*. Since the two plants

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are both tropical sclerophyllous evergreens, originally inhabiting rain-forests, this is not perhaps surprising. The most marked characteristic of the records is the large and abrupt changes in transpiration rate. The data for each

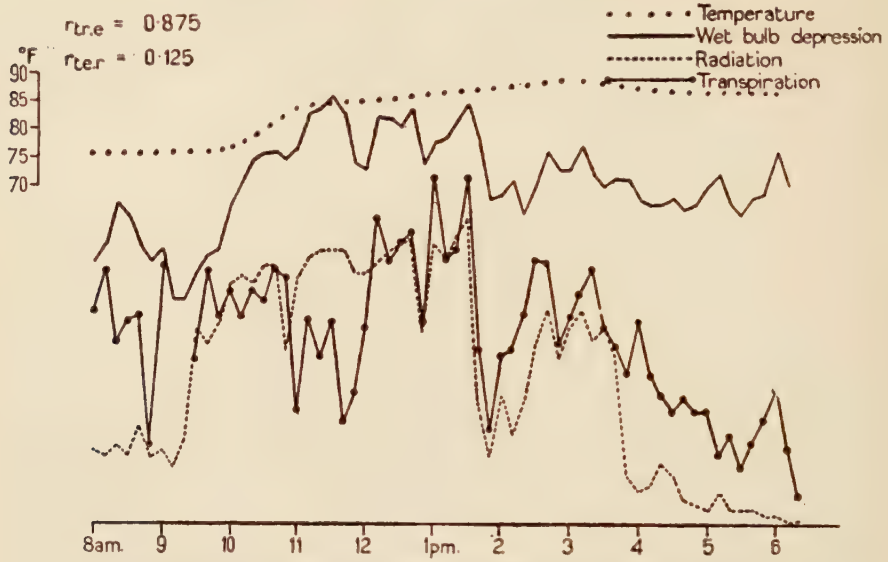

FIG. 1. March of transpiration, radiation, wet bulb depression, and temperature.

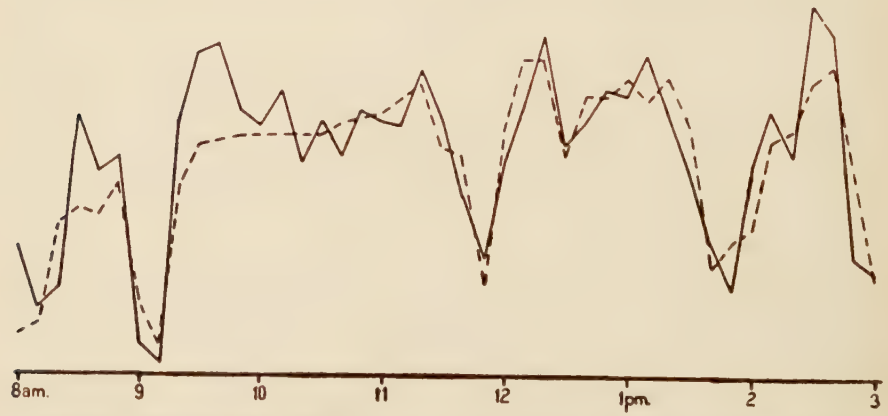

FIG. 2. March of transpiration rate [entire line] and solar radiation [broken line] for *Eugenia aromatica* on 19/1/49.

separate day have been analysed separately, and the results are summarized in the following table.

It is quite clear that the fluctuations in transpiration rate are very closely related to radiation, and, under the conditions of this work, are not caused by changes in the evaporating power of the air. Similar fluctuations in the transpiration rate of the coffee plant were shown (Nutman, 1941) to be due to

stomatal control of transpiration, stomatal aperture being affected by incident radiation. In coffee, moderate intensities of radiation result in stomatal opening and high transpiration; high intensities in stomatal closure and reduced transpiration. There is, therefore, a marked midday stomatal closure in coffee with a midday drop in transpiration. With the clove tree, no such midday drop in water-loss has been observed. This may be due either to the stomata not closing in light of high intensity or to the very large proportion of leaves in the clove-tree which are shaded by others at any given time.

TABLE I

Correlations between the Transpiration Rate of Eugenia aromatica (T) Solar Radiation (R) and Wet-bulb Depression (E), all Data being recorded as 10-minute Averages throughout the Day.

Date.	$r_{Tr.}$	$r_{TE.}$	$r_{RE.}$	$r_{TR.E.}$	$r_{TE.R.}$
24/1/49	**0·640**	**0·351**	**0·640**	**0·875**	−0·125
25/1/49	**0·707**	*0·273*	**0·409**	**0·695**	0·318
27/1/49	**0·660**	0·112	0·092	**0·677**	0·055
2/2/49	**0·883**	−0·286	**−0·457**	**0·852**	0·060
4/2/49	**0·684**	*−0·322*	0·068	**0·714**	0·417
5/2/49	**0·666**	**0·412**	*0·324*	**0·618**	0·277
8/2/49	**0·698**	*0·248*	**0·356**	**0·711**	0·001
9/2/49	**0·712**	0·173	−0·036	**0·742**	−0·211
10/2/49	**0·674**	*0·345*	**−0·482**	**0·742**	0·032

Coefficients significant to the 1% point are printed in bold-face type and those to 5% in italic type.

Unfortunately the structure of the clove leaf is such that the usual methods of recording stomatal apertures cannot be used. Some indirect evidence as to their behaviour is available from the study of assimilation rates of single horizontal clove leaves. The method used was to pass a stream of ordinary atmospheric air through one tube of an infra-red gas analyser, then through a perspex assimilation chamber attached to the underside of the leaf, and then through the second tube of the analyser. The air-stream was so adjusted that the change in carbon-dioxide content never exceeded 10 per cent. of normal.

Figure 3 illustrates a typical result, obtained on a hot sunny day. The leaf was shaded by others when recording was started and assimilation rose fairly steadily during the morning. It was in full sun at 10·50 and assimilation fell sharply, rising again during a cloudy period from 11·30 to 11·45. From 2·20 the leaf was increasingly shaded by others and assimilation again increased.

In a horizontal leaf of *E. aromatica*, therefore, there is evidence that high light-intensity causes a reduction in assimilation rate. While there is no direct evidence as to the cause, analogy with *C. arabica* makes it seem possible that stomatal control is being exerted, especially since rough field-studies, using the injection technique, suggest that midday closure is usual in exposed clove leaves in full sun, whereas shaded ones have open stomata.

The recorded transpiration rate of a whole clove-tree, however, does not support this conclusion. Nutman (1950) has presented evidence to show that stomatal control of transpiration rate is effective in the clove leaf. The explanation may, as previously suggested, lie in the dense bushy habit of the

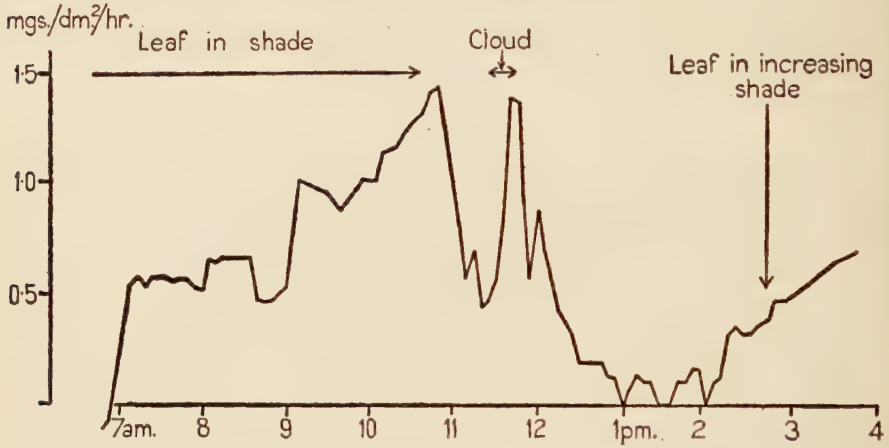

FIG. 3. Apparent assimilation rate of a single horizontal attached leaf of *Eugenia aromatica*.

clove plant, whereby a high proportion of the leaves are in shade. The high transpiration rates of these might compensate for the reduced transpiration of the leaves in full sun.

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Dimorphism and Monomorphism in the Plumbaginaceae

III. Correlation of Geographical Distribution Patterns with Dimorphism and Monomorphism in Limonium

BY

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With two Figures in the Text

Abstract

In the normally self-incompatible *Limonium*, as in the related genus *Armeria*, there appears to be an important positive correlation between establishment after long-distance dispersal and self-compatibility. Thus a seed-reproducing colony may be produced in a self-compatible species even though only a single disseminule overcomes the hazards of long-distance dispersal. With this in mind, the distribution and possible migratory routes of various sections of the genus are traced from the Old World. Particular attention is paid to Atlantic and amphi-Atlantic distributions (in relation to both 'Continental Drift' and 'Permanent Continent' theories of recent geological history) and also to the special problems of *Limonium* in Australia.

Introduction

THE previous paper in this series (Baker, 1953) contained a tabulated survey of the pollen and stigmata of species in all the sections and subsections of the genus *Limonium* as it is understood at present. Comments were made upon the possible phylogenetic importance of the findings and on their consequent value for any future taxonomic revision of the genus. There are also several features of considerable phytogeographic interest revealed by the survey, and demonstrable correlations exist between reproductive method (as revealed by the pollen- and stigma-morphology) and geographical distribution. In considering these, little reference will be made to the subsections Densiflorae and Dissitiflorae in which many species are apomictic (cf. Baker, 1950). Similarly, the sections containing species with capitate stigmata have been considered before (Baker, 1953).

The overall distribution of the genus embraces all five continents (see map in Baker, 1948) and is certainly that of an ancient group of species. The sections and subsections with relatively continuous distributions present few problems for solution; these are found where there are large disjunctions. Consequently, attention will be focused on the latter group first.

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1. Sections with Disjunct Distributions

(*a*) *Atlantic and amphi-Atlantic distributions*

Sections Pteroclados and Ctenostachys

The section Ctenostachys contains species from Morocco, the Canary Isles, and the Cap Verde Islands. This is a natural, although disjunct, distribution. At present there are some 50 miles of ocean between Morocco and the nearest of the Canaries, over 1,000 miles from the Canaries to the Cap Verde Islands, and some 400 miles between these last islands and the nearest part of the African mainland (which is many miles south-west of Morocco). Nevertheless, all of the species are dimorphic and form a morphologically well-knit section, and there are resemblances in habit between these species and those of the equally dimorphic section Pteroclados (with its subsections Odontolepidae and Nobiles). The group of nine species forming the Nobiles is restricted to the Canary Isles. They are shrubby (which might be construed as an ancient character) and their endemic nature, combined with the known fact that the distributional area of the species has decreased and is decreasing, even to the point of extinction (Stapf, 1906), supports the view that the Nobiles are an old-established group. In the largely Mediterranean subsection Odontolepidae, *L. thouinii* (Viv.) Kuntze is of particular interest because of its distribution from the Middle East through the Mediterranean lands (particularly on the North African side) to the Canary Isles. Despite the widespread distribution, including the large Atlantic disjunction, the species has kept its dimorphism of pollen and stigmata. All of the species examined (in both sections) are homostylous.

Section Limonium

Subsection Genuinae. The distribution of the dimorphic and monomorphic species of this large subsection is extremely clear. The dimorphic species are characteristic of the salt-marshes of Europe and salty places in the steppes of Asia, and are also to be found in salt-marshes on both the east and west coasts of South America. The monomorphic species are, with only one exception, North American. The monomorphism is, without question, of the secondary type; the combination of type A pollen with papillate stigmata being found as usual.

There can be no doubt that the original home of this subsection is in the Old World. Apart from the fact that the great majority of the sections and species of *Limonium* are Old World in distribution, so that a New World origin is unlikely, the concentration of monomorphics in North America points to a derived condition (just as in *Armeria* (Baker, 1949)). A very important question which may be raised is how, when, and where *Limonium* of this subsection entered the New World. Distribution across the Pacific Ocean, even across the Bering Straits, is unlikely as the subsection does not reach the Far East of Asia.

The dimorphic, self-incompatible *Limonium vulgare* Mill. is found, not only

around the Mediterranean and west European shores, but also in the Azores. There is some dispute as to the number of forms or species in the Azores (cf. Drouet, 1866; Godman, 1870), but they certainly include this one. This might indicate the route to South America. Although chromosome-counts will not be considered in detail here, it is interesting that the subspecies of *L. vulgare* which is common to southern Europe and the Azores (*L. v.* subsp. *macrocladon* Salmon = *S. serotina* Reichb.) appears to contain diploid and tetraploid forms while the typical northern European material of the species is tetraploid ($2n = 36$) (Baker, unpub.). The chromosome-numbers of the South American representatives of this subsection are not yet known, but a comparison of pollen-sizes with those of known diploids and tetraploids in the subsection suggests that they, too, are diploid. *L. brasiliense* (Boiss.) Kuntze, the northernmost representative on the Atlantic coast of South America, is not unlike *L. vulgare* in many morphological respects. It is dimorphic and, as Muller pointed out as long ago as 1868, it is heterostylous, as is the European species. '*Statice uruguayensis* Arech.' and *L. patagonicum* (Speg.) Macl. are probably best placed as subspecies of *L. brasiliense*. They are both dimorphic and serve to carry the distribution-range of the dimorphic forms right to the southern extremity of the continent.

On the Pacific side of South America, *L. guaicuru* Kuntze (*S. chilensis* Phil.) is also dimorphic and probably related (cf. Wangerin, 1911). There are many floristic connexions between Chile, in the south, and Mexico and California north of the tropics (cf. Johnston, 1940; Wulff, 1943; Campbell, 1944; Constance and Shan, 1948; Stebbins, 1950; &c.) and, although there is no population of *Limonium* yet found along the more than 5,000 miles of coastline which cross the equatorial belt between these regions, *L. mexicanum* Blake and *L. californicum* (Boiss.) Heller (which are found on the Pacific coasts of Baja California and California proper, respectively) are very like the Chilean species in general morphology. However, a significant change has occurred in pollen and stigma correlation and in the breeding-system. Both of these very closely related species are monomorphic (with type A pollen and papillate stigmata). Living material of both species has been examined; both are diploid ($2n = 18$) and experiments have shown them to be completely self-compatible.

It is unlikely that the gap between the North and South American sea-lavender populations has ever been continuously bridged and some long-distance dispersal will have been necessary. Therefore it is interesting to see that *L. californicum*, itself, has a disjunct distribution. Although the main distribution of the species is along the Californian coast from San Diego in the south to Humboldt County in the north, it is also to be found in a trio of stations in saline washes in the deserts on the Nevada side of the Californian border (Blake, 1923; Tidestrom, 1925). These situations are more than 200 miles removed from the coast and it is inconceivable that they can ever have been connected with the coast by a continuous population or even that such a population could have moved gradually inland. The conclusion seems

justifiable that these stations have been colonized after relatively long-distance dispersal, and it is in this that the significance of the self-compatibility of these plants may be seen. A seed-reproducing colony may be produced even though only a single disseminule successfully overcomes the hazards of long-distance dispersal from the coast to inland desert.

A similar picture is shown by the related species, *L. limbatum* Small, of New Mexico and Texas. This is another monomorphic species which is found in scattered saline washes separated by great expanses of relatively inhospitable desert. It is close enough to *L. californicum* for Tidestrom and Kittell (1941) to place it under that species in their 'Flora of New Mexico', its chief mark of distinction being a flaring calyx.

L. limbatum serves to link the Pacific coast *L. californicum* with the species along the coast of the Gulf of Mexico. From Tamaulipas in Mexico, along the coastline of the Gulf States of the U.S.A., around the Florida peninsula, and as far north along the Atlantic coast as Labrador, is to be found a series of 'species' of *Limonium* which belong to this subsection. From pollen-measurements it seems that they are all tetraploids (whereas all previously mentioned New World species of this subsection appear to be diploids). These 'species' are greatly in need of experimental study, having been described largely from herbarium material (Wangerin, 1911; Blake, 1916). Intermediates between several of them have been found and the limits at which the specific boundaries have been drawn seem to be arbitrary even after Blake's (1923) consolidation of his previous list (cf. also Fernald, 1950). Nevertheless, the great quantity of material of these species which is to be found in the larger herbaria of the U.S.A. and in England has been examined (including types or topotypes in every case) and, each time, monomorphism of the type A/papillate kind has been found, even in the specimens intermediate between one so-called species and another.

Considerable significance is to be attached to the fact that some material from the northern end of the range is so much like the European *L. humile* Mill. that specimens have been originally given that name. Conversely, these monomorphic, self-compatible species show a greater morphological divergence from *L. californicum* as the geographical distance from California increases. The most obvious trend is towards a greater separation of the spicules so that, in the extreme, *L. nashii* Small var. *trichogonum* (= *L. trichogonum* Blake), from the northern Atlantic seaboard, shows well-separated spicules such as are found in the European *L. humile*. There are no intermediate teeth between the lobes of the calyx in *L. mexicanum* and *L. californicum*, but these are present in the Atlantic and Gulf Coast species, and here *L. limbatum* provides the link, possessing well-defined teeth.

A very striking outlier of this group is found in *L. lefroyi* Hemsl. which is endemic to the islands of Bermuda, some 700 miles into the Atlantic Ocean from the North American mainland. One is tempted to see here the successful long-distance dispersal of a monomorphic ancestor from the American coast, for it has never been seriously suggested that the Bermudas are anything but

oceanic islands. The same explanation seems likely for the presence of *L. humile* in Europe. This species, as mentioned previously, is morphologically very close to the North American forms, and its localized European distribution is such as to cause Matthews (1937) to place it in his 'Oceanic Northern Element' of the British Flora along with such famous North American-European species as *Eriocaulon septangulare*, *Spiranthes romanzoffiana*, *Juncus dudleyi*, *J. tenuis*, &c. (see also discussion by Heslop-Harrison, 1952). *L. humile* fits into this picture perfectly by being the only European species of the genus to be monomorphic with type A pollen and papillate stigmata, just as in its North American relatives. Its styles are shorter than the stamens; a feature of all monomorphic species of this subsection and a derived condition from the heterostylous dimorphic species.

L. vulgare and *L. humile* have retained their inter-compatibility, and the hybrid between them is not uncommon where the species meet in the salt-marshes of northern Europe. This hybrid (*L. neumani* Salmon) is fairly fertile and, by means of pollen- and stigma-studies along with considerations of general morphology, it has been possible to demonstrate that not all natural hybrids are of the F_1 generation.

The order in which the species of this subsection have been treated here is suggested as an indication of the most likely path of migration from the Old World to the New and then back again, and is illustrated diagrammatically in Fig. 1.

The monomorphic *L. humile* is found both in Ireland and Great Britain but, while the dimorphic *L. vulgare* is quite a common species in English, Welsh, and Scottish salt-marshes, it is apparently unable to cross the Irish Sea and colonize Irish salt-marshes. This apparent inability can hardly be due to climatic factors, for the eastern Irish coast enjoys a climate less extreme in either direction than the Welsh or eastern English coasts, respectively. Thus while monomorphic self-compatible species appear to be consistently able to carry on a 'stepping-stone' method of distribution (forming colonies which contain plants reproducing by seed even after what looks like long-distance dispersal), the same ability does not characterize dimorphic species.

Of all migrations of this subsection, the west-to-east crossing of the Atlantic would seem to be the most recent if we remember that the monomorphic species involved are probably younger than the original dimorphic stocks and, also, that so far only one relatively uncommon species has been produced after the European re-immigration and that this species has not diverged morphologically very far from its North American congeners. On the other hand, we have postulated that the crossing of the Atlantic Ocean from the Old World to South America by the dimorphic ancestors began this tale of migration and therefore took place at a much more remote period. Yet the dimorphic species seem to be ill adapted for ocean-crossing, and, as will be seen later, when a more complete picture of the genus has been drawn, there are at least six characteristically dimorphic sections or subsections which have reached remote groups of Atlantic islands and one is endemic therein. This

suggests that, at such an early time, the Atlantic Ocean was by no means the barrier to migration that it is today.

Wegener's much-discussed hypothesis of the geological history of the world claims that the Atlantic Ocean is of relatively recent origin (Wegener, 1929; Du Toit, 1937; Wulff, 1943; Hutchinson, 1946). This would allow North

FIG. 1. Diagrammatic map of the distribution of species of the subsection Genuinae in Europe and the Americas with indications of likely migration paths. Dimorphic species in capitals; monomorphic species in lower case.

Africa and South America to be in direct connexion until the Eocene period, while the breadth of the ocean would have been relatively small for some further time. The Eocene is not too early for the beginning of the very long series of migrations postulated above, especially when it is considered that there is evidence that the genus *Limonium* in the wide sense may be even older (p. 624). It need not be assumed that the centre of variability of the genus was then, as it is now, in the Mediterranean region (cf. Maury, 1886), although it seems almost certain to have been in the western part of the Old World.

If migration to South America took place in such circumstances, it might have been expected that a similar migration would have taken the genus

directly from Europe to North America. Nevertheless, as explained above, the evidence is against this having occurred. It may be that as the world's past climatic fluctuations become better known we shall see some likely reason why no member of the genus was in a position to make such a crossing until the gap had become too wide for dimorphic species to cross successfully.

As an alternative to the Wegenerian hypothesis just outlined, the possible significance of the present distribution of the genus must be considered against a history of relative permanence of continental positions. In this case the representation of dimorphic species both in the Atlantic islands and on both sides of the South Atlantic Ocean might only mean that the distribution of seeds of the ancestral species had gone on for a very long time. The establishment of a type A and a type B plant in close enough proximity for cross-pollination to take place eventually occurred and permitted reproduction by seed in the new situations. The return crossing by the derived monomorphic stock could still have been successful in the shorter available time because of the advantage of self-compatibility in establishment after crossing.

Subsections Steirocladae and Hyalolepideae. Examination of a great deal of material has convinced me that these subsections cannot be kept distinct from each other, and it is noteworthy that no author since Boissier has maintained any such separation. Consequently they will be treated here as a single group. The species of this widespread, probably ancient group, are characteristically dimorphic. The greatest concentration of species is to be found in the Mediterranean region, whence the distribution through the Middle East and southern Russia even as far as north-eastern Asia is a natural one and contains no remarkable disjunction. Similarly, the extension of the group southwards from the Mediterranean to Arabia on one hand, and through the Sahara to the coast of West Tropical Africa on the other, is not difficult to comprehend phytogeographically. However, there are large disjunctions between these species and those in the West Indies (*Limonium bahamense* (Griseb.) Britton and *L. haitiense* Blake) and between the main concentration and the considerable group in southernmost Africa. In both cases there are dimorphic species on both sides of the disjunction. All of the South African species which have been examined have shown dimorphism of pollen and stigmata and the same is true of *L. bahamense*. These facts seem to confirm the considerable age of the group; that it is old enough either for long-distance dispersal to have been achieved successfully despite the handicap of self-incompatibility or for a once-continuous distribution to have become broken up. The South African group appears to have been isolated long enough for considerable local evolution to have occurred.

Nevertheless, monomorphism (A/papillate) and presumable self-fertility is shown by *L. haitense* which is very closely related to *L. bahamense*. Such monomorphism does coincide with a disjunction (between the Bahamas and the island of Haiti), although this is not of comparable magnitude with the major ones in the group. Notes on the taxonomy and detailed distributions of this pair of species will be published elsewhere.

Limonium cosyrense (Guss.) Kuntze, from the islands of Sicily and Pantellaria, has been shown to be apomictic (Baker, 1953). The acquisition of apomixis may, however, be relatively recent, and it is not possible to draw any phytogeographical conclusions from its demonstration in a single species covering a relatively small area.

Limonium billardieri (Gir.) Kuntze was included in the subsection Steirocladae by Boissier (1848). Unfortunately it has not been possible to examine any material of this species and, indeed, its reputed distribution—restricted to the single island of Bouru, in the Moluccas, with no relative closer than South Africa, some 6,000 miles away—is so remarkable that one wonders if some mistake may not have been made in the origin of the type material. There is a precedent for this in the case of '*Statice tetragona* Thunb.' (see p. 623).

Section Jovibarba

This monotypic section lies near to the section Limonium (cf. Pax, 1897). *L. jovibarbum* (Webb) Kuntze, its species, is found only in the Cap Verde Islands. It has dimorphic pollen and stigmata. This species may represent a natural divergence from a common ancestral stock with the section Limonium in the isolation of island life. Divergence of sufficient magnitude has occurred, however, for one to suppose that the isolation is of relatively long standing. This accords with the general picture of dimorphic species inhabiting Atlantic islands.

Limonium echioides.

This species is the only one with the self-compatible combination of type B pollen and 'cob' stigmata (Baker, 1953), although many plants (particularly from Cyprus) are male-sterile. Its geographical distribution is worthy of study. Because it has been shown that *L. echioides* must be removed from association with *L. cabulicum* and *L. owerinii* in the section Schizhymenium, there is no significance in the lack of coincidence in their relative natural areas. *L. echioides* is found in all of the countries which fringe the Mediterranean from the Middle East to Morocco and Spain, and in the islands. Not only does it occur in coastal localities but inland in separated saline washes. It is tempting to see here a correlation between self-compatibility or apomixis and distribution-pattern such as was suggested for *L. californicum* and *L. limbatum*. There may also be a correlation between the apomixis or self-compatibility of *L. echioides* and its annual habit (which is unusual in the genus).

(*b*) *Oriental distributions*

Section Plathymenium

Subsection Rhodantheae. This east-central Asiatic group seems to be generally dimorphic. It has not been possible to examine enough material of *L. congestum* (L.) Kuntze to see whether this species is truly monomorphic or not, but it should be placed on record that all eight pollen-determinations which have been made revealed type B pollen.

Subsection Chrysantheae. An interesting feature of the pollen of this subsection is the large size of the grains. The group is characteristically East Asiatic; the bulk of the species (*L. aureum*, *L. bicolor*, *L. chrysocomum*, *L. schrenkianum*, and *L. xantholeucum*) centre around Mongolia. Hemsley (1902) records *L. aureum* (L.) Baker (inedit.) from 13,363 ft. above sea-level in Tibet. Nevertheless, all of these species are dimorphic. *L. sinense* (Gir.) Kuntze, which is found farther south than any of those previously mentioned (reaching Hainan Island and in Formosa), is very closely related to the Japanese and Korean representative, *L. japonicum* (Sieb. et Zucc.) Kuntze. So close is this resemblance that Bentham and von Mueller (1869) united both of these taxa with the eastern Australian species *L. australe* (Spreng.) Kuntze, under a common name. *L. australe* is distributed along the coast from Queensland to Tasmania and also occurs in New Caledonia, nearly 1,000 miles from the nearest point in Queensland. Nevertheless, while *L. sinense* is dimorphic, *L. japonicum* and *L. australe* are monomorphic with type A pollen and papillate stigmata. Undoubtedly this monomorphism is secondary and its acquisition may have been extremely important in the successful crossing of the very considerable sea-barrier separating the Chinese and Japanese species from that in Australia, a barrier which can never have been bridged by land (see below). The remaining species, *L. wrightii* (Hance) Kuntze (*S. arbuscula* Maxim.), ranges from Formosa through the Riu Kiu Islands almost to Japan proper, and this species has preserved its dimorphism.

When he created this subsection Boissier (1848) added '*Statice tetragona* Thunb.' to it. This was described as a species from the Cape of Good Hope, South Africa. Inquiry after material of this species in the herbarium at Kew prompted Mr. A. A. Bullock, of that institution, to make a closer study of its identity. The results of this investigation (Bullock, 1949) show clearly that the locality given on Thunberg's herbarium sheet (now at Uppsala) is erroneous and that the specimen is identical with *Limonium japonicum*. Unfortunately this means the replacement of the name *Limonium japonicum* (Sieb. et Zucc.) O. Kuntze by *L. tetragonum* (Thunb.) Bullock for the Japanese and Korean species, but in the present paper it has seemed desirable to use the more familiar name. It also means, however, that an extraordinary apparent disjunction no longer needs explanation.

Limonium in Australia

Despite the probable Malaysian origin of many of the characteristic plant groups of Australia (Diels, 1934), it is generally believed that the land-connexion between Australia and the island-groups of Malaysia (excepting New Guinea) was ended in the Upper Cretaceous period. Nevertheless, even by that time, many families usually reckoned to be quite highly evolved appear to have been in existence in Australia (Suessenguth, 1950).

There is further evidence (Suessenguth, 1950) of a land-link between Australia and Patagonia through a more temperate Antarctica as late as the Eocene, although, as Stebbins (1950) points out, this may not have been a

continuous bridge, but rather a series of 'stepping-stones' with the Antarctic continent forming the largest land-mass. It is important to note that it is only *western* Australia which seems to have been concerned in this, being separated from eastern Australia by a sea-barrier which was not bridged until the Miocene. Hooker (1860) noticed that endemic species are commonest in the south-western maritime areas and least common in the tropical north. In post-tertiary time the Antarctic continent has been too cold and distant to supply new species to Australia, and it is only from the north that temporary land-bridges and long-distance dispersal can have conveyed additions to the flora (Suessenguth, 1950).

Within the Plumbaginaceae, three genera (*Plumbago*, *Aegialitis*, and *Limonium* (sensu lato)) possess Australian species and, in *Limonium*, with which we are concerned, these are classified in two widely separated sections. In the first place we may consider the phytogeographical significance of the relationship between the South American section Pterolimon and the Western Australian section Arthrolimon which seems to be indicated by the similarities in their pollen-morphology. The relationship cannot be close, as their abundant general morphological differences emphasize, but it appears likely that they are the remnants of an ancient stock. It is significant that the two Australian species (*L. salicornaceum* (F.v.M.) Kuntze and '*Statice macphersoni*' F.v.M.) are restricted to the extreme west of the continent (Fig. 2), and we may therefore visualize the migration of some type ancestral to the present-day sections in one direction or the other between South America and Western Australia at a time not later than the Eocene and probably much earlier. Because of the overwhelmingly greater representation of *Limonium* in the Old World than in the New, one may suspect that the passage was from Australia to South America rather than in the opposite direction. Both sections consist of halophytic species, so the migration seems likely to have occurred either along a shore-line or else across arid country.

Limonium australe, which belongs to the relatively advanced section Plathymenium and resembles so closely Japanese and Chinese species, must be a relatively recent immigrant, and this is in agreement with its distribution from Queensland to Tasmania (Fig. 2). It has already been pointed out (p. 623) that its monomorphism and consequent self-compatibility must have been of great assistance in establishing the species after such a huge ocean-crossing. Only a similar explanation can account for the presence of this species in New Caledonia, nearly 1,000 miles away from the eastern Australian coast.

2. Sections with Continuous Distributions

Section Limonium

Subsection Sarcophyllae. The species of this morphologically well-knit subsection are distributed from Sind to Arabia and Egypt through Persia and to the Aegean through the Caucasus. A tongue runs through Somaliland and reaches the Comoro Islands in the Indian Ocean. All the species which have

been examined have proved to be dimorphic in pollen and stigmata. Maury (1886) has suggested that it is in this subsection that the genus *Limonium* approaches closest in morphology to *Limoniastrum*, and there is a geographical coincidence also. Dimorphism of pollen and of linear stigmata is also found in *Limoniastrum*, where, however, the bases of the styles are fused.

FIG. 2. Approximate ranges of *Limonium salicornaceum* (●), '*Statice macphersoni*' (×) and *Limonium australe* (thick lines) in Australia.

Sections Sphaerostachys, Polyarthrion, Myriolepis, and Siphonantha

All species of these tiny sections which encircle the Mediterranean possess dimorphic pollen and stigmata. In addition, *L. caesium* (Gir.) Kuntze, *L. ornatum* (Ball) Kuntze (sect. Polyarthrion), and *L. ferulaceum* (L.) Kuntze and *L. diffusum* (Pourr.) Kuntze (sect. Myriolepis) have been proved to be self-incompatible.

Section Psylliostachys

These tiny-flowered steppe herbs have a relatively tight Middle Eastern distribution, apparently centred in Persia. They are dimorphic in pollen and stigmata. Self-incompatibility has been proved in *L. suworowii*. It is especially remarkable that three out of the four species examined are annuals (where self-compatibility is the general rule in flowering plants). *L. leptostachyum* (Boiss.) Kuntze, in particular, is extremely diminutive. In each of the species, dependence is upon the dense inflorescence rather than the individual flower for insect-attraction.

Summary

1. Secondary monomorphism of pollen and stigmata has arisen in the subsections Genuinae, Steirocladae/Hyalolepidae, Chrysantheae of the genus *Limonium*, and in *L. echioides*. In each case this is correlated with a disjunct distribution. The significance of such monomorphism (as in *Armeria*) appears to be in the facilitation of the establishment of a seed-reproducing colony after immigration from a distance. This suggests that a general survey of the relation between self-compatibility and distribution-patterns in the flowering plants is worth while.

2. A possible migration route for the subsection Genuinae is given. In it, the occurrences of dimorphism and monomorphism give point to the morphological and cytological trend from Europe around South America to Pacific North America and then back to Europe via the Atlantic States. *L. humile* appears to culminate a series which began with a form resembling *L. vulgare* var. *macrocladon*. All considerations point to an Old World origin for the genus, but the results offer no positive evidence for or against 'Floating' or 'Permanent Continent' theories of geologically recent history.

3. In the Far East a rather similar pattern presented by the Asiatic subsection Chrysantheae shows the monomorphic disjunct species *L. australe* to be a relatively recent immigrant into Australia and New Caledonia. By contrast, the apparently ancient Western Australian section Arthrolimon shows modest affinity with the South American section Pterolimon.

4. Sections with restricted, more or less continuous distributions show only dimorphism of pollen and stigmata.

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An Analysis of Turgor and Turgor Pressure

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With one Figure in the Text

ABSTRACT

Turgor depends on the excess pressure inside a cell, not upon a reaction between the wall and contents. It is independent of the environmental pressure whereas the total reaction is not. Turgor pressure is not identical with the reaction, or, unless the effects of the protoplasmic membranes be ignored, with the wall pressure. Changes in turgor pressure can be said to cause changes in volume, but only when both are actually simultaneous results of differential diffusion. The effects of environmental pressures are considered. Some misconceptions are discussed and some terms more fully defined.

A WIDESPREAD uncertainty suggests that it is desirable to formulate the method of operation of turgor pressure in conferring rigidity and to clarify the relation between it and some of the associated quantities, especially perhaps the reaction between the cell wall and cell contents.

Turgor is rigidity resulting from distension. Rigidity implies that work must be done to cause distortion. The work involved is that required to stretch the cell envelopes (wall and membranes) when the cell or tissue is bent. The wall and membranes will only become stretched when the cell (or tissue) is bent if conditions are such that the wall on the outer (convex) side of the bend is maintained at a distance from the wall on the inner (concave) side of the bend. In other conditions (i.e. if there is nothing to keep them apart) stretching is evaded by the walls approaching one another and the formation of a kink. The resistance to bending and therefore the degree of turgor depends upon the moment of the forces developed in the envelopes on the convex side of the bend about the corresponding point on the concave side where the material is under compression. It is therefore greater (*a*) the greater the force set up in the envelopes by bending, and (*b*) the greater the distance apart maintained between the opposite walls. The walls are only kept apart by the excess pressure inside the cell over that outside. Turgor therefore depends (*a*) upon an increased tension being produced in the cell envelopes on one side on bending (and therefore work being done upon them), and (*b*) upon an excess internal pressure keeping the envelopes apart and so maintaining the moment of forces developed in the envelopes on one side about the other side. It is this moment which actually resists bending and decides the amount

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of work necessary to cause it. Theoretically at least all the cell envelopes must contribute in some small measure to the force developed, but undoubtedly in practice the contribution of the cellulose cell-wall is by far the most important. The cell has been likened by Thoday (in private correspondence) to an H-section beam, the cell-wall withstanding the tensile forces like the flanges and the contents playing the part of the web in keeping the flanges apart. In the cell the wall is there all the time and potentially capable of withstanding tensions if they can be developed: the variable factor is the equivalent of the web. Turgor, like the resistance to bending of a beam, depends on the contents and the wall, the web and the flanges, acting together; when the cell is flaccid the walls are allowed to approach one another by failure of the web, and it is on this account that there is no resistance to bending. In practice, therefore, the essential condition for turgor is mere fullness of the cell (since slight bending would then set up a slight internal pressure militating against further mutual approach of the walls) or for higher values an excess pressure inside the cell over that outside to keep the walls apart. The degree of turgor may therefore be measured in terms of this excess hydrostatic pressure. Its *modus operandi* is by distancing the opposite walls so that more work has to be done in stretching the envelopes on one side on a given deformation.

It will be observed that in describing the way in which turgor pressure confers turgor or rigidity no reference has been made, or need be made, to any reaction between any two envelopes of the cell or between one envelope and the cell contents. Such a reaction, perhaps contrary to widespread ideas, is in fact in itself no essential for the condition of turgor and is purely incidental. Except if the environmental pressure be either zero or entirely supported by an incompressible wall so that it is not communicated to the cell contents, and even then only if the protoplasmic membranes support the whole of the internal pressure, a reaction is a necessary concomitant of the essential condition for turgor, an excess internal pressure, but turgor in no sense depends on the reaction, and the value of the reaction is no measure of the degree of turgor. This is especially evident from the consideration among others that whatever one's conception of the nature of the tension in the protoplasmic membranes, and whether these be considered as fluid or semi-solid, tensions in the protoplasmic membranes will always reduce the reaction between the protoplast and the wall, but, since they increase the excess internal pressure and therefore the work done in distortion, must increase the degree of turgor.

Turgor then does not depend upon the existence of a reaction as such either between two envelopes or between one envelope and the contents. A single envelope (e.g. a rubber balloon) can possess the property of becoming more rigid on distension and can therefore have turgor. Two envelopes are not necessary.

When two or more envelopes act together as in a plant cell, the effective quantity is the total excess pressure inside as offset by the sum total of all the tensions in all the stretched envelopes acting together, *not* the reaction

between any two. A reaction between the envelopes is of no importance as such: the only essential in determining degree of turgor is an excess internal pressure to keep opposite walls apart and so to maintain a high moment of forces in the wall under tension about the corresponding point in the wall under compression.

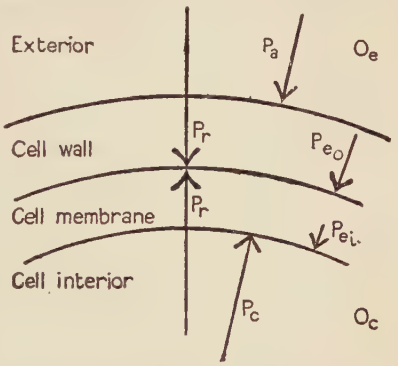

FIG. 1

The following analysis helps to settle difficulties which arise in connexion with the use and significance of some of the terms in common use when the effects of the environmental pressure and tensions in protoplasmic membranes are taken into account. The form of the analysis will be made similar to that of Sven Algeus (1951) with which, however, as will emerge, the writer is not in complete agreement.

To simplify the analysis all envelopes other than the cell wall (including the inner and outer protoplasmic membranes and the protoplast itself) potentially capable of contributing to the pressure in the vacuole by being in a state of tension have been grouped together as 'the membrane'. They could obviously be considered separately on the same principles if information concerning their individual properties and conditions warranted this treatment. (Sven Algeus only considered the contribution of the outer protoplasmic membrane, although the inner is probably osmotically the more important.) 'Membrane' in what follows must therefore be taken to include all envelopes between the wall and vacuole capable of existing under elastic or surface tension. Their effects on the hydrostatic pressure inside the cell can only be additive, so separate symbols for them would be superfluous. Separate symbols for different osmotic potentials in different regions enclosed by different membranes would also be superfluous since only the region with the highest osmotic equivalent (Levitt, 1951*b*) would be effective in deciding turgor and others would come into equilibrium with it by mutual volume adjustments.

Consider (Fig. 1) an element of cell wall and its associated membrane as presented by Sven Algeus (1951).

Let the surrounding environmental pressure (atmospheric pressure, or hydrostatic pressure round a submerged cell) acting inwards on the outer wall $= P_a$

Let the pressure exerted by the wall on the membrane as a result of elastic stretch in the wall only $= P_{e_o}$

Let the total pressure exerted inwards on the membrane $= P_r$

Let the total pressure exerted outwards by the membrane on the wall (reaction to P_r above) $= P_r$

Let the pressure exerted by the membrane on the cell contents as a result of the elastic stretch in the membrane only $= P_{e_i}$

Let the hydrostatic pressure inside the cell, measured at the surface of the protoplast[1] $= P_c$

Let the osmotic potential of the external solution be (or the surrounding atmosphere have a saturation deficit equivalent to) $= O_e$

Let the osmotic potential of the cell contents $= O_c$

Then, the inward pressure of the wall on the membrane, being due partly to stretch in the wall itself and partly to the environmental pressure acting through the wall

$$= \text{the centrifugal } P_r = P_a + P_{e_o}{}^2 \tag{1}$$

and the outward pressure on the wall by the membrane (being due to the pressure in the cell lessened by the stretch in the membrane)

$$= \text{the centripetal } P_r = P_c - P_{e_i}, \tag{2}$$

and since the centrifugal and centripetal mutual reactions are necessarily equal and opposite (P_r out $= P_r$ in)

$$P_a + P_{e_o} = P_c - P_{e_i}. \tag{3}$$

It may be noted that the writer's main criticism of Sven Algeus (1951) is that he appears not to recognize these three fundamentals, (1), (2), and (3), and consequently treats the reaction as if it were independent of the other pressures mentioned instead of consisting in the sum of them.

Three useful restatements of (3) give

$$P_c - P_a = P_{e_o} + P_{e_i} \tag{4}$$

$$P_{e_o} = (P_c - P_a) - P_{e_i} \tag{5}$$

and

$$P_c = (P_{e_o} + P_{e_i}) + P_a \tag{6}$$

Hence, from (4) the excess hydrostatic pressure inside the cell = the sum of the pressures set up by the elastic stretch in the wall and membrane;

from (5) the pressure on the membrane due to stretch in the wall is the excess hydrostatic pressure inside less any pressure due to stretch of the membrane; and

from (6) the hydrostatic pressure inside is the sum of the pressures set up by stretch in the wall and membrane plus the environmental pressure.

All these statements appeal also to common sense. It remains to assign definite meanings to the conventional terms 'hydrostatic pressure of the cell contents', 'wall pressure', and 'turgor pressure', the P_c, P_w, and P_t respectively of Levitt (1951*a*), in terms of the more fundamental symbols used above. This will be done with regard to the originally intended implications of the terms.

The 'hydrostatic pressure of the cell contents' is naturally, as seen from

[1] During changes in volume there is a radial gradient in hydrostatic pressure (cf. Spanner, 1952).

[2] Concerning dynamic states, see p. 635.

equation (6), the environmental pressure increased by the pressures due to stretch in the wall and membrane. At equilibrium it is uniform throughout the cell, but in dynamic states, while water is passing radially into or out of the cell, must, as shown by Spanner (1952), exhibit a radial gradient. It is evident from the considerations on p. 630 that the effective value deciding degree of turgor must be some mean value of the internal pressure, not the maximum or minimum value measured very near to the membrane. The value of P_c concerned in the above equations can only be that at the surface of the fluid contents immediately inside the membrane, but since the radial gradient can only at the most be very minute and almost purely theoretical, and since there can be no means of measuring P_{c_s} at the surface of the cell as distinct from P_{c_c} at the centre, it will be sufficient for purposes of these considerations and for all practical applications to identify the P_c of Levitt with the P_c of the equations, i.e. with P_{c_s}, although strictly speaking it should be identified with some mean of P_{c_s} and P_{c_c} as being the exact quantity deciding the degree of turgor. The 'hydrostatic pressure of the cell contents', P_c, will then be taken as the total internal pressure measured at the surface of the fluid cell contents immediately under the membrane. (It may be noted that actually the P_c of Levitt is apparently measured relative to the atmospheric pressure and therefore less than the P_c defined above by P_a. Such a convention is adequate for most purposes but not for the considerations of the effects of environmental pressure given below.)

Concerning the term 'turgor pressure' it is obviously logical and reasonable to take this as the pressure which makes for turgor. Turgor, as shown on p. 630, depends only on the excess hydrostatic pressure inside the cell as compared with that outside, so turgor pressure must, as shown by equation (4), be identified with $P_c - P_a$ and therefore with the sum of the pressures due to elastic stretch in the wall and membrane, $P_{e_o} + P_{e_i}$. It must not be identified with the reaction between the wall and membrane, P_r, nor with the reaction between the membrane and fluid contents, $P_{r'} = P_r + P_{e_i}$, $= P_c$, since, as shown by equation (1), the reaction, P_r, and therefore also the reaction, P_{r_i}, depend also upon P_a which does not assist in producing the condition of turgor. (This point will be discussed further below; see p. 635.) From these considerations it is definite that

$$\text{Turgor pressure} = P_t = P_c - P_a \tag{7}$$

$$= P_{e_o} + P_{e_i} \tag{8}$$

$$= P_r + P_{e_i} - P_a. \tag{9}$$

It cannot therefore be identified with the reaction between the wall and the contents unless P_{e_i} be ignored and all pressures be measured relative to atmospheric pressure. For many purposes there is nothing against these simplifications, but in many circumstances they will not give a complete or accurate picture of the conditions so that the more complete form of the equation is essential. (Cf. the argument on pp. 635–7.)

Concerning the term 'wall pressure', it appears logical and reasonable to

define this as the inwardly directed pressure due to and set up by the elastic stretch in the wall only. It is therefore identifiable with P_{e_o}. Hence

$$\text{Wall pressure} = P_w = P_{e_o} = P_r - P_a \text{ (from (1))} \tag{10}$$

$$\text{which from (5)} = P_c - P_a - P_{e_i} \tag{11}$$

Wall pressure cannot therefore be identified either with the reaction or with the hydrostatic pressure of the cell contents, unless again P_{e_i} be ignored and pressures are measured only relative to atmospheric pressure. This simplification may be allowable for most purposes, but as in the preceding case does not always give a complete or correct picture of the circumstances, so the full equation should be used.

Summarizing:

Turgor pressure is the excess hydrostatic pressure inside the cell and may be taken as the difference between the hydrostatic pressure immediately inside the membrane and in the environment.

Wall pressure is the pressure exerted by the forces due to elastic stretch in the wall only.[1]

The hydrostatic pressure inside the cell is the environmental pressure plus the total pressure due to stretch in the wall and membrane. This equals the reaction between the membrane and fluid cell contents.

The reaction between the membrane and fluid cell contents is the environmental pressure plus the turgor pressure.

The reaction (most usually considered) *between the wall and membrane* is less than this latter reaction by the pressure due to stretch in the membrane, so is given by

$$P_r = P_a + P_c - P_{e_i}. \tag{12}$$

All these quantities are distinct. Even when P_{e_i} is ignored the reaction between wall and membrane is greater than the hydrostatic pressure inside the cell by the environmental pressure, but in this case the turgor pressure or *excess* pressure inside becomes identical with the wall pressure. These generalizations and equations (1) to (12) above are true of all cases in static states.

The conditions for equilibrium may now be considered. At equilibrium with an outside solution of osmotic potential, O_e, or an atmosphere with a saturation deficit equivalent to this, the

Diffusion potential of water inside the cell = Diffusion potential of water outside the cell

or

$$D_{H_2O} - O_c + P_c = D_{H_2O} - O_e + P_a. \tag{13}$$

Substituting for P_c from (6)

$$D_{H_2O} - O_c + P_{e_o} + P_{e_i} + P_a = D_{H_2O} - O_e + P_a \tag{14}$$

or

$$O_e = O_c - (P_{e_o} + P_{e_i}). \tag{15}$$

[1] See below (p. 635) concerning dynamic states. Hygen and Kjennerud (1952) refer to the total reaction, P_r, as the wall pressure.

If P_{e_i} is neglected $O_e = O_c - P_{e_o}$ (16)

$= O_c - P_w$ (17)

$= O_c - P_t$. (18)

It is to be noted that neither the reaction, P_r, nor the environmental pressure, P_a, comes into this equation or contributes to conditioning the equilibrium. (The whole argument may if preferred be stated vectorially (as by Levitt, 1951*a*) without affecting the results.)

Concerning non-equilibrium or dynamic states, as clearly stated by Hygen and Kjennerud (1952), during expansion or contraction of the cell the reaction of the wall on the contents consists of three components, one due to environmental pressure, a static one due to elastic tension in the wall, and a dynamic one (comparatively very minute) due to its inertia. It is the sum of all these, or total reaction, which is at all times exactly equal to the reaction of the contents P_r. The total wall pressure, P_w, then consists in the static P_{e_o} due to stretch plus a minute inertia component, P_{w_i}. Equation (10) would be rewritten

$$P_w = P_{e_o} + P_{w_i} = P_r - P_a \qquad (10')$$

The excess pressure of the contents, $P_r - P_a$ or $P_t - P_{e_i}$, though still equal to the total wall pressure, P_w, is minutely different from its static component by the amount of the inertia component, P_{w_i}, being minutely greater than P_{e_o} during swelling and minutely less during contraction. It is this difference, P_{w_i}, which constitutes the 'driving force' of swelling. To cover all static and dynamic states precisely, the wall pressure, P_w (cf. p. 634), should be defined as the inwardly directed pressure due to the elastic stretch in the wall only, plus (when operative) a component due to the inertia of the wall. Rewriting equation (10) as (10′) above, the fundamental equations (cf. equations (1), (2), and (3)) would become

$$P_r = P_a + P_w \qquad (1')$$

$$P_r = P_c - P_{e_i} \text{ (unaltered)} \qquad (2)$$

and since $P_w = P_{e_o} + P_{w_i}$, $P_a + P_{e_o} + P_{w_i} = P_c - P_{e_i}$. (3′)

These equations and any derived from them (as by writing $P_{e_o} + P_{w_i}$ for P_{e_o} in equations (4) to (8)) will *always* be true for *all* states. The value of the inertia component, P_{w_i}, however, can only be so infinitesimally small compared with P_{e_o} that this adjustment is only of theoretical importance.

It is of interest to note that some considerations of supposed analogies might at first appear to suggest that the environmental pressure should be taken into account as affecting the degree of turgor and therefore the turgor pressure. These, however, on analysis only go to show the dangers and limitations of some of the frequently used partial analogies. That of the football will illustrate the point.

If a half-inflated football is placed in a vacuum pump receiver and the pressure lowered, the bladder will gradually expand since the air inside will

occupy a larger volume at the lower pressure and the ball will become 'turgid'. This experiment incidentally illustrates the principle mentioned earlier (p. 630) that the environmental pressure (the original atmospheric pressure in this case) opposes the tendency of the internal pressure to keep the walls apart, and therefore militates against turgor.

Similarly, if a fully inflated football has the environmental pressure increased, e.g. by taking to the bottom of the sea, it will become flaccid. The case and bladder being deformable, the pressure inside will become the same as the environment pressure as soon as the environmental pressure is great enough to compress the enclosed air to the volume of the unstretched bladder. The air in it at any higher pressure will not occupy sufficient volume to allow it to fill the case. (It is assumed that the unstretched bladder fits the unstretched case.)

On the other hand, a turgid plant cell at the bottom of the sea could be brought to the surface (reducing the environmental pressure as in the first football experiment above) without any permanent change in its turgor. If the experiment were performed slowly there would be no change. If performed too quickly for immediate readjustment and supposing the cell was originally in a condition of incipient plasmolysis, the initial pressure inside would be the same as that outside, but during the reduction of external pressure on ascent the originally slightly compressed cell contents would slightly expand; this would slightly stretch the wall and temporarily set up a small turgor pressure. This would increase the potential of the water inside and cause just enough water to pass out to re-establish the original condition of incipient plasmolysis. The degree of turgor would be the same, and the only difference would be that the cell would contain minutely less water at the lower pressure and more at the high pressure owing to the compressibility of the water. Similarly, if the original cell were plasmolysed or turgid, its re-equilibrated condition would also be the same as the original condition, assuming only in all cases that the osmotic concentration of the surrounding solution remained the same. For instance, if originally fully turgid, on bringing up to the surface the pressure inside and outside would become less: the originally more compressed water inside would expand, increasing the stretch of the wall and the turgor pressure. This would then force water out and the wall would contract again until the original size and equilibrium were reached, and the original pressure difference between the inside and outside was re-established. If a turgid cell at the surface were taken down, the pressure would increase both inside and outside, the contents would become slightly more compressed, and the volume minutely less. The wall pressure would be temporarily reduced and a small amount of water would pass in until the original equilibrium was regained. Similar arguments hold for cells exposed to atmospheres of constant saturation deficit and variable barometric pressure, providing only that the cells be originally in actual equilibrium with the humidity of the air and only the pressure is altered.

The cases cited above and similar ones may be proved from the equations.

The last example, of a turgid cell at the surface being taken down into the water, may be taken as an illustration.

Suppose a partially turgid cell in equilibrium with sea-water at the surface is transferred to a depth in the medium.

Let the original values of the internal and external osmotic potentials and the internal and external hydrostatic pressures be respectively $= O_c$, O_e, P_c, and P_a, and the original turgor pressure ($= P_{e_o}+P_{e_i}$ if both envelopes are considered) be written for simplicity as $= P_t$ (cf. (8)).

Then (cf. (18)) $O_c = O_e+P_t$ (19)

and $P_c = P_a+P_t$. (20)

On transferring the cell to a depth in the liquid and increasing the environmental pressure

Let the new external or environmental pressure be $P_a+\Delta P$.

Let the immediate reduction in volume, before any readjustment by flow of water has taken place be ΔV

the original volume $= V$

and the immediate new volume on compression $= V-\Delta V$.

Then before any flow of water has taken place

the internal osmotic potential becomes $O_c \times V/V-\Delta V$,

the turgor pressure becomes $P_t-\Delta P_t$

the internal hydrostatic pressure becomes $P_a+\Delta P+P_t-\Delta P_t$.

The diffusion potential of water outside $= D_{H_2O}-O_e+P_a+\Delta P$

and the diffusion potential of water inside the cell

$$= D_{H_2O}-O_c \cdot V/(V-\Delta V)+P_a+\Delta P+P_t-\Delta P_t.$$

For equilibrium these must be equal, or, omitting common terms,

$$-O_e = -O_c \cdot V/(V-\Delta V)+P_t-\Delta P_t \quad (21)$$

or

$$O_c \cdot V/(V-\Delta V) = O_e+P_t-\Delta P_t. \quad (22)$$

But from (19) $O_e = O_c-P_t$.

Hence for equilibration, substituting for O_e in (22),

$$O_c \cdot V/(V-\Delta V) = O_c-\Delta P_t. \quad (23)$$

But since $V/(V-\Delta V)$ is necessarily >1 when ΔV is positive, and since ΔP_t cannot be negative when ΔV is positive (i.e. since turgor cannot increase as the volume decreases), the only possible solution to this is that

$$V/(V-\Delta V) \rightarrow 1$$

and

$$\Delta P_t \rightarrow 0.$$

Equilibration therefore consists in ΔV and ΔP_t both $\rightarrow O$, or the changes

in volume and turgor disappear. Since both volume and turgor have been reduced this indicates that water must flow into the cell until the original volume and turgor have been restored. The cell therefore comes back to the original equilibrium with the same values of osmotic potential and turgor pressure, but these will only be reinstated by a small amount of water flowing into the cell. The other cases mentioned are susceptible to similar treatment: there is never a permanent change in turgor as a result of altering the environmental pressure.

The environmental pressure, therefore, although altering the total reaction between the cell wall and cell contents (cf. (1)), does not affect the turgor pressure. Turgor, turgor pressure, and wall pressure are all, after adjustment has been made by flow of water, unaffected by the environmental pressure. The time required for the adjustment must depend upon the permeabilities of the cell envelopes to water.

It may be noted that the reasons for the difference between the behaviours of the cell and the football in this respect are that in the football the changes in internal pressure depend only on the different volumes in the different conditions of a fixed quantity of air, the bladder being impermeable to air, whereas in the cell the changes are accompanied by changes in the quantity of the contents, the envelopes being freely permeable to water. The essential difference is that air does not pass in and out of the football but that water does pass in and out of the cell. The analogy is therefore not by any means complete for many circumstances and must be used with caution.

Three statements of Sven Algeus call for comment.

The first is that turgor only depends upon the value of p in his diagram, the reaction between the wall and contents. This arises from a misconception concerning the way in which distension confers rigidity. It has been shown (pp. 629–30) that turgor (rigidity resulting from distension) depends only on an excess pressure inside the cell maintaining the full diameter to resist bending and *not* upon the reaction as such. The reaction, p, is therefore immaterial; it is the excess pressure inside which decides the degree of turgor.

Secondly, Sven Algeus insists that the turgor pressure must not be confused with the pressure of the sap against the protoplast. This is bound up with the last and further emphasizes the same misconception that turgor depends in some unexplained way upon the reaction against the cell wall. Since it actually depends upon the total excess pressure inside the cell as increased also by the stretched membrane, the reaction between the sap and protoplast would be a better indication of the operative turgor pressure than that between the protoplast and the wall. A tension in the outer or inner protoplasmic membrane or both, though reducing the effect of the wall, would increase the turgor, not reduce it. On Sven Algeus' view a tension in the membranes would reduce turgor by reducing the reaction between the protoplast and the wall: actually, as shown on p. 630, a tension in the membranes would increase the turgor, and could even confer slight turgor in the absence of a wall altogether.

The third statement is that turgor pressure cannot be said to distend a cell. This is again bound up with Sven Algeus' definition of turgor pressure as the pressure of the protoplast against the wall, according to which it becomes identifiable with the reaction. Even on this definition, however, there is no reason why it should not cause distension. As the outwardly directed pressure within the cell it would be just as responsible for distension of the cell as the pressure in a football for the distension of the ball. It is immaterial that the wall pressure is equal to it since this is merely another expression of the same pressure. This internal pressure, though not the prime cause, is the immediate mechanical cause of distension, and as such, turgor pressure would be generally agreed to distent a cell and any increase in turgor pressure to increase distension. But it can only do so when it is itself in turn due to influx of water. In such cases, as in all ordinary cases of expansion of cells in dilute solutions, the real cause of distension is actually the inward movement of water resulting from a higher diffusion potential outside. This in turn brings about the increased turgor pressure and the corresponding increases in volume and wall pressure exactly simultaneously. Turgor pressure and extension are therefore perfectly simultaneous parallel results of differential diffusion of water, but, provided no lag is implied, the extension may be regarded as indirectly due to influx of water and immediately due to the resulting increase in pressure.

Different views on the mechanism of distension may easily result from different conceptions of the extensibility of the cell wall. If one visualizes the wall as having high elasticity and therefore only stretching with difficulty, one tends to think of the pressure as the first result of influx of water and distension as resulting from the pressure. If one visualizes the wall as stretching very easily, one may think of the increase in volume as occurring first and of this as involving further stretching of the wall, which in turn brings about higher elastic tensions in the wall, higher wall pressure, and thus the higher internal pressure in the cell. In one case the argument is that the turgor pressure causes the distension, in the other that the distension causes the increased turgor pressure. The views are equally true up to a point, but equally incomplete, and equally wrong in their implications that either change can precede the other. It is true that turgor pressure acting as an instantaneous intermediary must be the immediate cause of distension when this is actually due to influx of water, but the whole point is that distension and increase of turgor pressure are really two exactly simultaneous parallel consequences of the influx of water, due in turn to a difference in diffusion potentials inside and outside the cell.

Changes in volume will not run parallel with changes in pressure, however, when the changes in pressure are not due to influx or efflux of water. If, for instance, there should be an increase in the elasticity of the wall or membrane resulting from a change in imbibition or other conceivable cause such as a change in temperature, the elastic tensions at the given degree of extension would be increased. This would increase the turgor pressure. This in turn would increase the diffusion potential of water inside the cell and so cause

efflux of water and the cell would contract. (This is perhaps more readily realized if the increased elastic tensions are stated to increase the wall pressure, but the implication is exactly the same.) An increase in turgor pressure therefore causes contraction if it results from changes in the elasticity of the wall or membrane and *causes* movement of water, but causes distension when it is *caused by* movement of water into the cell. In the latter case, although it is the immediate cause of distension, both it and the distension are actually parallel and exactly simultaneous results of the influx of water.

Summary

Turgor (or rigidity due to distension) depends upon an excess pressure inside an envelope serving to keep the opposite walls distant from one another. This resists simple compression and also maintains a high moment of the forces in the wall under tension about a corresponding point in the opposite wall (the wall under compression) when a bending stress is applied. This is the necessary condition for resisting bending. Turgor is therefore not dependent upon a reaction between the envelope and its contents. It is shown to be independent of the environment pressure, whereas the total reaction between the cell wall and cell contents is not. Turgor pressure is not identical with the reaction, or, unless the effects of the protoplasmic membranes be ignored, with the wall pressure. Turgor pressure can be said to distend a cell, but only when the change in turgor pressure altering the degree of distension is due to influx of water. In such cases the changes in turgor pressure and the changes in volume are actually parallel and exactly simultaneous results of influx or efflux of water resulting from a difference in diffusion potential inside and outside the cell. If turgor pressure is increased by a change in elasticity of the wall or membrane it leads to contraction of the cell. The response of cells to changes in hydrostatic pressure and the failure of the analogy of the football in this respect are discussed. Some misconceptions have been analysed. The terms used have been more fully defined.

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